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RENAL MECHANISMS IN COINCIDENT ADDISON'S DISEASE AND DIABETES INSIPIDUS . . . 171
P. G. Skillern, A. C. Corcoran and A. L. Scherbel
ANALYSIS OF POLYURIA INDUCED BY HYPOPHYSECTOMY IN MAN . . 183
M. B. Lipsett, J. P. Maclean, C. D. West, M. C. Li and O. H. Pearson
RELATIONSHIP OF DIURNAL VARIATION IN 17-OH-CS LEVELS IN BLOOD & URINE TO EOSINOPHILS & ELECTROLYTE EXCRETION 196
R. P. Doe, E. B. Flink and M. G. Goodsell
ACTH RESISTANCE IN ACROMEGALY 207
I. Ishihara, T. Mukai and T. Tanaka
NATURE OF ADRENALINE & NORADRENALINE IN NORMAL HUMAN URINE 216
F. Elmadjian, E. T. Lamson and R. Neri
EXCRETION OF ADRENALINE & NORADRENALINE IN HUMAN SUBJECTS 222
F. Elmadjian, E. T. Lamson and R. Neri
GONADAL DYSGENESIS WITH ANDROGENIC MANIFESTATIONS IN THE TALL EUNUCHOID FEMALE 235
R. B. Greenblatt, N. Carmona and L. Higdon
USE OF METHYLTESTOSTERONE TO STIMULATE GROWTH 241
E. H. Sobel, C. S. Raymond, K. V. Quinn and N. B. Talbot
TREATMENT OF EXCESSIVE GROWTH IN THE ADOLESCENT FEMALE . . 249
M. A. Goldzieher
NONCONJUGATED ADRENOCORTICAL STEROIDS IN HUMAN PLASMA: COMPARISON OF TWO TECHNIQUES 253
C. J. Migeon, A. A. Sandberg, E. L. Bliss and A. R. Keller
BLOOD ACTH IN CHILDREN WITH RHEUMATIC FEVER 262
A. B. Brill, R. S. Ely, A. K. Done, L. E. Ainger and V. C. Kelley

TEACHING CLINIC
Use of Laboratory Tests in Diabetes. *F. D. W. Lukens* 272

LETTERS TO THE EDITOR
Thyroxine-Binding by Sera of Pregnant Women. *J. T. Dowling, N. Freinkel and S. H. Ingbar* 280
Urinary 17-OH-CS Patterns: Effect of ACTH & "Operative Stress." *N. I. Gold, D. A. Macfarlane and F. D. Moore* 282
Fluctuation in Excretion of Adrenocortical Steroids in Cushing's Syndrome. *G. Birke, L.-O. Plantin and E. Diczfalusy* 286
Comments on Rat Hyperemia Pregnancy Test. *R. L. Berman* 290
Effects of Chlorpromazine on Pituitary Function. *W. Z. Polishuk and S. Kulcsar* 292
Concerning the Term "Pseudo-pseudohypoparathyroidism." *G. A. Fromm* . . 293
THE ENDOCRINE SOCIETY. The 1956 Annual Meeting and Awards 296
THE AMERICAN GOITER ASSOCIATION. The 1956 Annual Meeting 299
The American Geriatrics Society. The 1956 Annual Meeting 299
Fourth International Congress of Internal Medicine 299
Regional Meeting—International Fertility Association 299
Book Register 300

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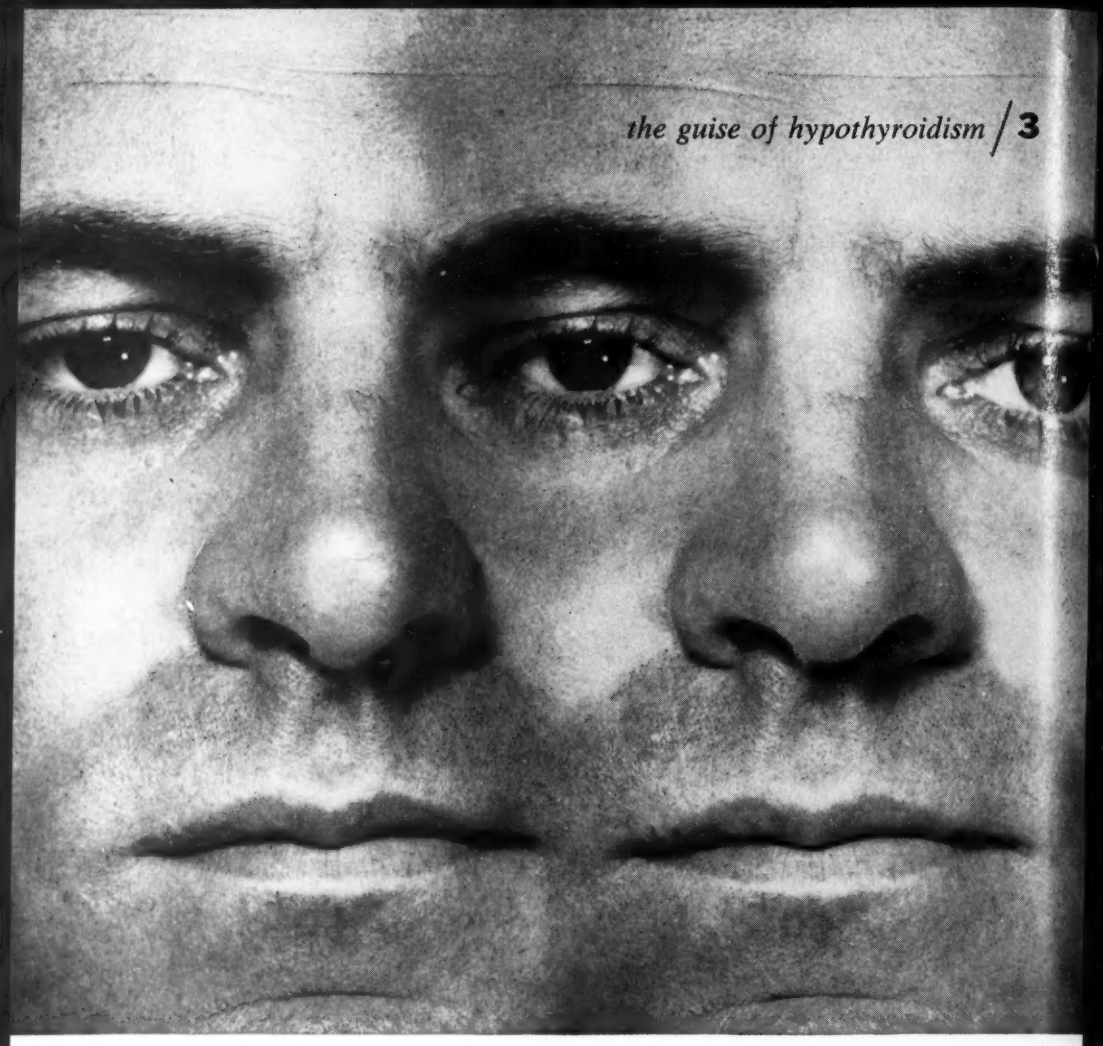

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RENAL MECHANISMS IN COINCIDENT ADDISON'S DISEASE AND DIABETES INSIPIDUS: EFFECTS OF VASOPRESSIN AND HYDROCORTISONE*

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INTRODUCTION

CONCURRENT primary failures of adrenocortical and posterior hypophyseal functions, with coincident syndromes of diabetes insipidus and Addison's disease, to our knowledge, have not been described heretofore. The case reported here has clinical interest, therefore, in that the concurrence is uncommon. It also has physiologic interest, in that the defects of water excretion characteristic of the two syndromes are, in some respects, disparate. These renal dysfunctions were demonstrated in our patient and the effects thereon of adrenocortical (hydrocortisone) and posterior hypophyseal (vasopressin (Pitressin)) hormones were determined. The data make it seem unlikely that the adrenal hormone has a specific action on renal tubular reabsorption of water.

CASE REPORT

A 51-year-old woman of Italian birth was admitted to the Cleveland Clinic Hospital on June 12, 1951. She had been weak, anorexic, thirsty and polyuric for the preceding

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four months, and during this time she had observed increasing skin pigmentation and a 19-pound loss of weight. She had not experienced nausea, vomiting, hiccup, or menstrual irregularity. A month before admission, her physician had given her obstetrical pituitary extract (1 or 2 doses of 1 ml. daily); following each injection there was a decrease of thirst and polyuria for several hours; for example, a dose at night decreased the frequency of nocturia from six or more times to once or twice.

On physical examination, she seemed asthenic. Body weight was 128 pounds. The skin showed extreme diffuse, grey-brown pigmentation, hyperpigmentation of the palmar creases and breast areolae, and very dark lentiginous spots on the feet and the forearms. There were spots of bluish pigment in the buccal mucosa. Blood pressure was 110/65 (left) and 110/70 (right arm) mm. Hg. The chest, abdomen and sella turcica were roentgenographically normal.

Serum sodium, potassium, and chloride levels were respectively 135, 4.6, and 96 mEq. per L., and carbon dioxide capacity was 24 mEq. per L. The blood urea level was 33 mg. per 100 ml.; hemoglobin, 13 Gm. per 100 ml.; red blood cells, 4,500,000 per cu. mm.; white blood cells, 5,400 per cu. mm.; and erythrocyte sedimentation rate, 1.7 mm. per min. (Rourke-Ernstene method; normal, less than 0.5). A differential white blood cell count indicated neutrophils 43 per cent, monocytes 2 per cent, and lymphocytes 55 per cent.

Pituitary extract was discontinued on admission, whereupon thirst and polyuria promptly recurred. Specific gravities of urine samples collected at that time were reported as 1.011 and 1.015, and that of a specimen obtained a week later, as 1.013. Two days after admission, the 24-hour volume of urine was 4,750 ml. A Robinson-Power-Kepler water test showed an eight-hour, night urinary volume of 1,050 ml. and, after ingestion of 20 ml. of water per Kg. of body weight, hourly volumes of 30, 20, 37 and 55 ml., in that order. Results of a tolerance test were within normal limits, and there was a normal urinary excretion of gonadotropin. However, urinary 17-ketosteroid levels on two occasions were only 1.5 and 2.0 mg. per twenty-four hours respectively (normal 5 to 11 mg.). The eosinophil counts before and after intramuscular administration of ACTH were 159 and 197 per cu. mm., respectively. Skin biopsies of lentiginous spots showed numerous dark-brown granules in varying concentrations in basal cells and few or none in the outer layers—a distribution consistent with that in Addison's disease.

The definitive diagnosis made at that time was Addison's disease; the diagnosis of associated diabetes insipidus was suspended because of the reported values for urinary specific gravity and the relatively low 24-hour volume of urine. The patient was started on a program of 4 mg. of desoxycorticosterone sublingually (2 mg. twice daily), 6 Gm. of sodium chloride daily, and a normal diet. Her condition became better and was further improved when, six weeks after the initial examination, the regimen was augmented by 12.5 mg. of cortisone daily. On August 28, 1951, she was pleased by her increased strength and appetite, but still had persistent polyuria and polydipsia; the daily doses of sodium chloride and desoxycorticosterone were reduced, respectively, by 2 Gm. and 2 mg.

When she was seen again in February 1953, she weighed 140 pounds. She said she felt well except for polyuria and polydipsia and some weakness. The menses had stopped abruptly in June 1952 and had not recurred. The blood pressure was 130/88 mm. Hg. She was advised to continue the same medication, and to use posterior pituitary powder (Armour) intranasally three times daily and at bedtime. This controlled the thirst and polyuria, so that the 24-hour volume of urine was stabilized at about 1,500 ml.

On March 26, 1953, a Robinson-Power-Kepler test was performed. Cortisone was omitted on the day of the test, but pituitary powder was taken. The night volume of urine was 770 ml.; after the water load the serial hourly urine volumes were 35, 135, 225, 140 and 110 ml. At that time, intramuscular administration of ACTH elicited no eosinopenia; a preinjection count was 144 per cu. mm., and four hours after injection the count was 131 per cu. mm. However, results of an intravenous saline test were consistent with the presence of diabetes insipidus (Fig. 1). The patient was advised to continue the regimen but to increase the cortisone dosage from 12.5 mg. daily to 25 mg. twice daily.

In September 1953, she said that she had been feeling completely well while following

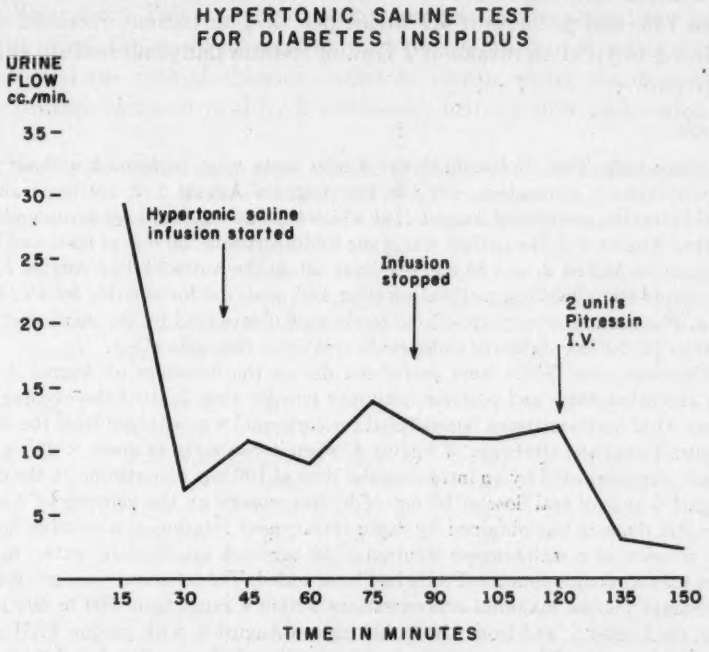

FIG. 1. Intravenous hypertonic saline test during a period of medication with cortisone only, in a patient having Addison's disease and diabetes insipidus. There was no decrease in diuresis until Pitressin in saline was given.

this program. The cutaneous pigmentation had diminished and the buccal spots had disappeared. She observed that when she neglected her nightly dose of pituitary powder, she was awakened by thirst and polyuria, but did not experience anorexia or weakness; however, when she also omitted cortisone, both anorexia and weakness recurred. She has since continued in this satisfactory condition. No cause for the coincident glandular failures has been determined. Rickards and Barrett (1) noted the occurrence of idiopathic granulomatous disease simultaneously in the adrenal and pituitary glands; it may be that our patient was similarly affected.

RENAL STUDY

The patient was readmitted to the ward of the Research Division of the Cleveland Clinic for study of renal function on August 1, 1954, more than three years after initial admission.

She seemed in good health and showed moderate, generalized "tan" with hyperpigmentation of the skin creases. The blood level of urea was 24 mg., sugar 83 mg. and hemoglobin 13.8 Gm. per 100 ml. Hematocrit index was 43 per cent, and the white blood cell count was 5,200 per cu. mm. Plasma electrolyte levels were: carbon dioxide 22.7, chloride 101, sodium 140, and potassium 4.7 mEq. per L. The patient received a diet calculated to yield an intake of 2 Gm. of sodium daily; no sodium chloride was given.

Methods

1. *Water tests.* Two Robinson-Power-Kepler tests were performed without recent posterior-pituitary medication. For the first test, on August 1–2, cortisone also was withheld after the morning of August 1, at which time 25 mg. had been given orally. For the test of August 2–3, the patient was given hydrocortisone, 50 mg. at noon and 50 mg. at bedtime on August 2, and 50 mg. one hour before the water load on August 3. Urine was collected by indwelling urethral catheter and analyzed for chloride by Dr. Harriet Dustan. Plasma and urinary creatinine levels were determined by the method of Miller and Miller (2) for calculation of endogenous creatinine clearance (C_{Cr}).

2. *Clearance tests.* These were performed during the mornings of August 4 and 6. Water and other fluids and posterior pituitary powder were omitted the evening before the tests. Oral hydrocortisone (substituted for cortisone) was withheld from the morning of August 3 until the afternoon of August 4, when it was given in doses of 25 mg. every six hours, supplemented by an intramuscular dose of 100 mg. of cortisone on the evening of August 5 and an oral dose of 50 mg. of hydrocortisone on the morning of August 6.

Osmotic diuresis was obtained by rapid intravenous infusion of a priming dose and slower infusion of a maintenance solution of 10 per cent mannitol in water, to which sodium para-aminophippurate (PAH) had been added. The infusions were given at rates that brought plasma mannitol concentrations within a range from 200 to 400 mg. per 100 ml. on August 4, and from 350 to 490 mg. on August 6, with plasma PAH concentrations of from 2 to 3.8 mg. per 100 ml. Osmolarities of plasma (P_{Osm}) and urine (U_{Osm}) were measured with a Fiske Associates osmometer, the accuracy of which was checked by reference to standard sodium chloride solutions of 300 and 500 mOsm. per L. Plasma (P), and urinary (U) PAH and mannitol concentrations were measured respectively by the methods of Smith and associates (3) and of Corcoran and Page (4); outputs of sodium and potassium were measured by a Baird Associates flame photometer with an internal lithium standard. Urine was collected by urethral catheter and each urinary collection was terminated by insufflating air and manually compressing the bladder. On August 6, at the end of the second period of clearance urinary collection, a dose of 50 milliunits of vasopressin (Pitressin) was given by instantaneous intravenous injection, and enough vasopressin was added to the infusion fluid to maintain an infusion rate of about 5 milliunits per Kg. per hour.

Urinary volumes (V) and clearance calculations made from these tests were corrected for body surface to 1.73 sq. M. Plasma mannitol (C_M) and PAH (C_{PAH}) clearances were

conventionally calculated as UV/P; plasma samples were secured during alternate periods of urinary collection and mid-point plasma concentrations (P) were estimated by interpolation. Osmolar clearances (C_{Osm}) were calculated as $U_{Osm}V/P_{Osm}$; free water clearances (C_{H_2O}) were calculated from the differences between V and C_{Osm}. A negative free water clearance measures the volume of osmotically unbound water abstracted from tubular urine in the function of urinary concentration; it is equivalent to the function $T^c_{H_2O}$(5); a positive free water clearance indicates excretion of a moiety of solute-free water with resultant formation of hypotonic urine.

Results

1. *Water tests* (Table 1). Hydrocortisone therapy increased the volume of night urine, the speed of onset and the maximum rate of water diuresis; it decreased the rate of chloride excretion during water diuresis. During water diuresis without oral hydrocortisone, urinary flow and endogenous

TABLE 1. WATER DIURESIS TESTS DURING INTERRUPTION OF HYDROCORTISONE THERAPY AND AFTER ITS RESUMPTION

Test	Date 1954	Time (hrs.)	Urine vol. (ml.)	Urine vol. (ml./min.)	Per cent of load excreted	C_{Cr} (ml./min.)	Chloride output (mEq.)
I	Aug. 1	10:30 P.M.					
	Aug. 2	7:30 A.M.	410	0.43		61	40
		8:30 A.M.	115	1.9	8.8	96	12
		9:30 A.M.	200	3.4	24	107	15
		10:30 A.M.	390	6.5	54	109	16
		11:30 A.M.	400	6.7	85	89	12
		12:30 P.M.	315	5.3	109	71	10
		1:30 P.M.	300	5.0	132	78	10
		10:30 P.M.					
II	Aug. 3	7:30 A.M.	1350	1.40		93	51
		8:30 A.M.	150	2.50	11.5	133	8
		9:30 A.M.	100	1.67	19	97	6
		10:30 A.M.	450	7.50	54	114	8
		11:30 A.M.	350	5.80	81	106	4
		12:30 P.M.	625	10.4	129	85	5
		1:30 P.M.	470	7.8	165	91	4

Modified water tests, indicating: volumes and rates of flow of 8-hour night urine (10:30 P.M. to 7:30 A.M.) and of serial hourly specimens of urine after water loads of 20 ml. per Kg. body weight; the percentage of this load excreted over these hourly periods; the chloride output in each urine specimen; and endogenous plasma creatinine clearance (C_{Cr}). None of these is corrected for surface area, which was 1.56 sq. M. Hydrocortisone was withdrawn before test I and given during test II.

creatinine clearance (C_{Cr}) tended to vary together; the fact that this association was not present after administration of hydrocortisone, confirms the observations of Burston and Garrod (6); hydrocortisone also increased average levels of C_{Cr} and decreased the variability of this function.

2. *Clearance tests* (Table 2; Fig. 2). These tests were performed during hydropenia and osmotic diuresis; morning samples of urine prior to mannitol infusion were hypotonic.

The first period of the clearance test of August 4 was not considered rep-

TABLE 2. CLEARANCES IN OSMOTIC DIURESIS AND HYDROPENIA DURING INTERRUPTION OF HYDROCORTISONE THERAPY AND AFTER ITS RESUMPTION

Date 1954	Time* (min.)	V	C_{PAH}	C_M	C_{H_2O}	F.F.	U_{Osm}	$U_{Osm}V$	U_{Na}	U_{Cl}	U_K
		(ml./min./1.73 sq. M.)					(mOsm./L.)	(mOsm./min./ 1.73 sq. M.)	(mEq./L.)		
Aug. 4	Fasting						130		38		
	0–10	13.5	680	80	5.2	.13	200	2.70	40	37	2
	–20	12.4	460	68	4.8	.15	200	2.48	38	37	5
	–31	13.6	430	55	5.3	.13	202	2.75	42	42	4
	–41	14.7	480	50	4.9	.10	220	3.24		47	8
	–53	14.8			4.7		230	3.40		54	8
	–63	16.9			4.8		240	4.06		55	7
Aug. 6†	Fasting	3.16					116	0.36	5	5	13
	–20– 0	17.3			9.0		158	2.72	25	24	10
	0–10	15.4	575	94	5.9	.16	204	3.14	27	23	8
	–20	14.8	485	86	5.6	.18	206	3.04	28	26	10
	–31	4.9	368	92	–2.6	.25	506	2.50	32	38	27
	–41	4.4	395	79	–3.8	.20	622	2.76	36	48	38
	–51	5.6	435	69	–3.4	.10	540	3.04	37	52	37

* Time (min.) = Time in minutes between successive bladder emptyings.
V = Urinary volume.
C_{PAH} = Plasma PAH clearance.
C_M = Plasma mannitol clearance.
C_{H_2O} = Free water clearance.
F.F. = Filtration fraction.
U_{Osm} = Urinary osmolarity.
$U_{Osm}V$ = Urinary osmotic load.
U_{Na}, U_{Cl}, U_K = Urinary electrolyte concentrations.
† Vasopressin infused (*see* text) at 20 minutes in the test performed on August 6.

resentative; estimates of C_{PAH} and C_M were high compared with subsequent urinary collections. Such "high first periods" are commonly found in studies of patients during osmotic diuresis and may reflect a transient renal vasomotor response to expansion of plasma volume by the hypertonic infusion. Usually, when the equilibration period after priming injection is prolonged, as on August 6, this phenomenon was not observed. The estimate of C_{H_2O} in the urine collected on August 6 during equilibration prior to clearance measurement was high and probably artifactual. This estimate was made during changes in plasma mannitol concentration, and the blood sample taken during the first clearance period may not have

been representative. With these reservations, the data indicated the following:

(a) Hydrocortisone treatment increased C_{PAH} and, to a relatively greater degree, C_M. These increases were associated with increases of arterial pressure, which was 110/70 during the clearance periods of August

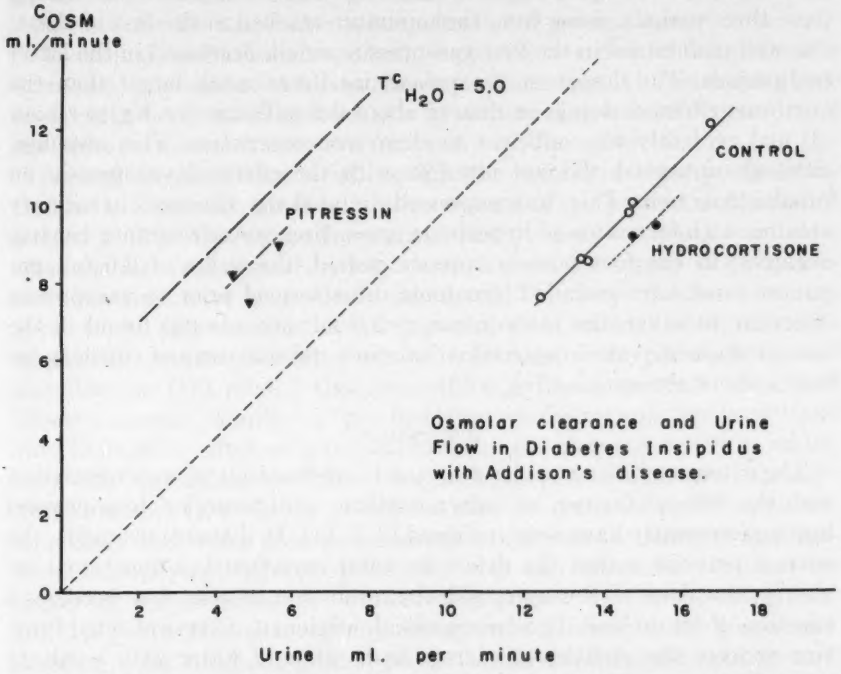

FIG. 2. Relationship between urinary volumes and osmolar clearances (C_{Osm}) during hydropenia and osmotic diuresis. The dotted line from the origin indicates isotonic excretion (no free water clearance). Lines are drawn (parallel to that of isotonic excretion) through open circles representing data from control observations without hydrocortisone or Pitressin, through the two closed circles representing observations after hydrocortisone only, and through two of the three closed triangles representing observations made after giving Pitressin. The dashed line at the upper left indicates the negative free water clearance ($T^c_{H_2O}$) at about the mean normal of 5 ml. per minute found by Zak, Brun and Smith (5).

4 and 122/82 during the first two periods of the test on August 6. Hydrocortisone also decreased both pre- and post-mannitol outputs of sodium and chloride; the decreases observed during osmotic diuresis were about 0.1 mEq. per minute, whereas potassium output was increased. The free water clearance (C_{H_2O}) of August 4 was positive and averaged 4.95 ml. per

minute, whereas the average of the two post-hydrocortisone, pre-vasopressin periods of August 6 was 5.75 ml. per minute.

(b) Vasopressin had an appreciable vascular effect. Arterial pressure increased from 138/86 just prior to injection, to a maximum of 150/96 at two minutes; it then fell to 110/70 at four minutes and was stabilized at about 130/70 during vasopressin infusion. C_{PAH} was decreased during these three periods, rising from the minimum reached in the first of these; C_M, well maintained in the first vasopressin period, decreased in the latter two periods. The dosage of vasopressin used was much larger than the minimum effective dosage in man of about 0.2 milliunit per Kg. per hour (3) and evidently was sufficient to elicit vasoconstriction. This response, although untoward, did not interfere with the effect of vasopressin on tubular function. This was expressed in a sharp decrease in urinary volume, with formation of hypertonic urine. Free water clearance became negative; in the first post-vasopressin period, the value of 2.6 ml. per minute must have included hypotonic urine formed prior to vasopressin injection; however, the rates (mean, −3.6 ml. per minute) found in the two subsequent periods were taken as minimum measures of capacity for free water reabsorption ($T^c_{H_2O}$) (5).

DISCUSSION

The extensive bibliographies relevant to mechanisms of water excretion and the effects thereon of adrenocortical and posterior hypophyseal hormones recently have been reviewed (3, 7–11). In diabetes insipidus, the current concept is that the defect in water excretion is a functional inability to reabsorb free water, with the result that urinary flow becomes a function of solute load. In adrenocortical deficiency, defects of renal function concern the abilities to excrete both salt and water with resultant "impaired *responsiveness* of the renal tubules to the homeostatic requirements of the moment" (12). The major defects in electrolyte handling consist of an impaired sodium reabsorption and diminished potassium excretion; these changes are attributed to deficiency of a direct tubular action of cortical hormone, notably aldosterone.

The gross defect in water excretion in adrenocortical deficiency is delayed excretion of an increased water load and has been attributed to various mechanisms. Some of these are either subsidiary or insignificant. Thus, slowed gastro-intestinal absorption is not a factor (13); hemodilution after water ingestion is only less prompt in patients having Addison's disease than it is in normal subjects (8); intravenous infusion of water does not elicit prompt excretion (13), and there is no reason to postulate a deficient responsiveness of osmoreceptors in Addison's disease. More

widely considered has been the attractive possibility of relative or absolute antidiuretic hormone (ADH) excess (7, 8). However, reports of cases of combined anterior and posterior hypophyseal failures, in which cortisone therapy had precipitated diabetes insipidus, indicate that ADH effect is not a major factor (14, 15), and the concept of an adrenocortical-ADH homeostatic balance seems specifically negated by the coincidence of Addison's disease and diabetes insipidus in the present case.

Two renal mechanisms have been suggested as causes of delayed water excretion in adrenocortical insufficiency. These are, 1) decreased glomerular filtration, and 2) absence of a hypothetical specific effect of adrenal hormone on water reabsorptive function.

Several considerations weigh against the second of these possibilities. One is the relatively normal diuretic response of our patient in the test of August 1, 1954; this was performed at a time (twenty to twenty-four hours after the last oral dose of hydrocortisone) when the supply of exogenous hormone must have been wholly exhausted. The same consideration may apply to the persistence for a few days of normal water diuretic responses in patients pretreated with cortisone intramuscularly, although Garrod and Burston (16) related this observation to the presence of depots of injected steroid. Another is the fact that cortisone and hydrocortisone have little or no effect on water exchange in the normal rat (17), in which they only potentiate the effect of simultaneously administered desoxycorticosterone. Lastly is the absence of effect of cortisone on urinary flow, osmolarity and water diuretic responses in the cases of anterior and posterior hypophyseal failures studied by Leaf and associates (18) and by Engstrom and Liebman (19). However, in support of a direct tubular action it is pointed out that salt and desoxycorticosterone restore glomerular filtration rates to about normal levels in adrenalectomized animals and Addisonian patients, but do not restore the promptness of water diuresis, whereas cortisone has both effects; moreover cortisone induces in dogs a diabetes-insipidus-like state which, unlike that caused by desoxycorticosterone, is not dependent upon salt intake (16).

The method of clearance study used in this case is adapted to measure any discrete effect of hydrocortisone on renal water reabsorption as a change in C_{H_2O}. The data indicate that this function increases by about 1 ml. per minute during hydrocortisone treatment. This increase is proportionately less than the concurrent increase in glomerular filtration rate and is roughly equivalent to the volume of water freed of electrolyte by increased sodium reabsorption. It is therefore attributable to increases either in the over-all nephron activity or in the availability of free water and not to a direct effect of hydrocortisone on renal water reabsorptive func-

tion. Hence, until, as Berliner (11) suggests "the relationship between filtration rate and electrolyte transport on the one hand and water diuresis on the other is clarified," we provisionally conclude that adrenocortical hormone has no direct specific effect on tubular water reabsorption and that, in relation to adrenocortical function, water diuresis is determined primarily by the glomerular filtration rate.

How, then, may the effect of hydrocortisone on water exchange be explained in this patient, whose 24-hour volume of urine without pituitary medication was 10 liters during hydrocortisone treatment in August 1954, in contrast with 5 liters in 1951 before treatment began? The observed effect of hydrocortisone on C_{H_2O} would account for only 1.5 liters daily. The probable explanation is that advanced by Stribling and Spurr (15) from their study of a patient with combined posterior and anterior hypophyseal deficiencies, namely that adrenal hormone indirectly stimulates thirst. We have seen that it increases electrolyte reabsorption and provides free water which, in the presence of the defect in water reabsorption created by posthypophyseal deficiency, is promptly excreted. The patient, unless she would emulate Lot's wife, must then drink water. Water ingestion, as in the patients of Stribling and Spurr (15) and Garrod and Burston (14), sustains and augments polyuria. This indirect effect is not observed when, as in the cases of Leaf and associates (18) and Engstrom and Liebman (19), the thirst mechanism as well as the posthypophyseal glandular mechanisms, is also defective.

After the administration of hydrocortisone to our patient, the responses to osmotic loads and to vasopressin corresponded with those of patients having diabetes insipidus alone (20). Based on the usual assumption of water excretion by filtration and reabsorption without vasopressin, the net defect in water reabsorption is about 9 ml. per minute. The negative free water clearance of 3.6 ml. per minute found after vasopressin is within the limits of normal for this function (5).

Lastly, the formation of hypotonic urine (for example on August 2, 1954 (Table 2)) raises doubt as to the accuracy of the measurements of urinary specific gravity made in 1951. The latter findings were important, in that they contributed to the deferring of the diagnosis of diabetes insipidus. Further doubt is cast on them by the consideration that, at a specific gravity of 1.010 and with a volume of urine of 5 liters daily, there would be excreted some 1,500 mOsm. of urinary solid, which is about twice this patient's probable daily rate. Some inaccuracy of urinometry may be presumed, but it should be recognized that decreased glomerular filtration and increased sodium and chloride outputs due to adrenal failure may have maintained urinary specific gravity at levels greater than those commonly seen in diabetes insipidus.

SUMMARY

1. A case of coincident cryptogenic diabetes insipidus and Addison's disease which responded to appropriate replacement therapy is described.

2. Cortisone and hydrocortisone increased water exchange and restored to normal the diuretic response to a water load; hydrocortisone increased the mean level and decreased the variability of endogenous creatinine clearance. The effect on water diuresis persisted longer than could be attributed to a direct action of the steroid hormone on renal tubules.

3. Clearance studies in osmotic diuresis and hydropenia showed that hydrocortisone increased renal plasma flow, glomerular filtration rate, and free water clearance. The small increase in the latter function is attributed to an indirect rather than to a direct action of the steroid on the specific tubular mechanism of water reabsorption. The defective water excretion caused by adrenocortical insufficiency is interpreted as a sequel to diminished glomerular filtration. Since the increase in free water clearance during steroidal therapy is too small to account for the observed large increase in water exchange, the latter is related to stimulation of thirst.

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AN ANALYSIS OF THE POLYURIA INDUCED BY HYPOPHYSECTOMY IN MAN*†

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DIABETES insipidus has been the subject of extensive animal investigations during the last twenty-five years, but the clinical counterparts of these studies have been few. This is due, not only to the rarity of the disease and its variable pathology, but also the considerable difficulty attending the diagnosis in its milder forms.

We have had a unique opportunity to observe the development of diabetes insipidus in several patients who have undergone hypophysectomy for metastatic cancer. We shall discuss the alterations in water exchange following surgery, and some of their physiologic implications.

METHODS

The studies were carried on the Metabolic Ward. The patients had advanced metastatic cancers, the majority of which were mammary carcinoma. Intake-output charts were kept for all the patients, both preoperatively and for extensive periods postoperatively. None of the patients to be discussed had any evidence of renal disease, when judged by such indices as the levels of blood urea nitrogen and serum creatinine or by the results of urinalysis and concentration tests.

The hypophysectomies were performed through two approaches. The patients of one group were operated on via a trans-antral, trans-sphenoidal route by Dr. C. C. Harrold of the Head and Neck Service of the Department of Surgery at Memorial Center. In this approach, no tissue above the diaphragma sellae was removed.

In the larger group of patients the operation was performed via a transtemporal or, currently, a transfrontal approach to the pituitary, by Dr. Bronson Ray, Head of the Department of Neurosurgery, New York Hospital, and Professor of Clinical Surgery,

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Cornell University Medical College. In this method, the stalk is cauterized and sectioned as close to the tentorium as possible, the pituitary is curetted and the pituitary fossa is zenkerized to ensure completeness of hypophysectomy.

Following surgery, the patients were catheterized and the urine output was followed at one-hour or two-hour intervals. Postoperatively, 5 per cent glucose in water was administered intravenously at a rate of 100 cc. per hour, and Pitressin tannate in oil was used when the urinary volume exceeded 3 cc. per minute for from two to four hours. Oral fluids were started when tolerated; this was usually on the first postoperative day. The catheter was removed and the intravenous fluids discontinued when the patient was able to take oral fluids *ad libitum*. Pitressin tannate was given only when the polyuria was severe. The patients were maintained following surgery on 37.5–50.0 mg. of cortisone acetate daily.

In the weeks following hypophysectomy, an attempt was made to assess the completeness of the procedure by determinations of 1) the level of protein-bound iodine, 2) the thyroidal uptake of I^{131}, 3) the urinary levels of follicle-stimulating hormone, and 4) vaginal smears when indicated. Several patients underwent withdrawal of cortisone or ACTH, in an attempt to provoke adrenal insufficiency. The hypophysectomy was considered to be functionally complete when the foregoing tests indicated severe hypofunction of the target glands. The sella turcica was examined grossly and microscopically at autopsy, when possible, although serial sections were not made. The effects of hypophysectomy on thyroid, adrenal and gonadal function will be reported separately. The hypertonic saline test, as described by Carter and Robbins (1), was performed on several patients.

RESULTS

Tables 1 to 4 are a résumé of the clinical data with respect to water exchange following hypophysectomy. The patients listed in these tables

TABLE 1. TRANS-SPHENOIDAL HYPOPHYSECTOMIES (8 PATIENTS)

Pt.	Completeness of hypoph'y		Survival postop. (days)	Immediate polyuria		Permanent polyuria; onset postop. (days)	Water exchange. No. of times preop. amt.
	By functional tests	At autopsy		Onset postop. (hrs.)	Urine flow (cc./min.)		
M.H.		Incompl.	9	None		None	1
M.F.		Compl.	3	4	8	1	—
L.B.	Incompl.		250*	None		None	1
M.Dol.		Compl.	32	2nd–3rd day	4	None	1
G.B.	Incompl.		450*	—		None	1
S.Sip.	Compl.		360*	None		None	1
J.R.	Compl.		96	None		None	1
W.G.		Compl.	12	None		None	1½

* Alive.

TABLE 2. COMPLETE HYPOPHYSECTOMIES (26 PATIENTS)

Pt.	Survival postop. (days)	Immediate polyuria		Permanent polyuria; onset postop. (days)	Water exchange. No. of times preop. amt.	Max. Sp. Gr. postop.
		Onset postop. (hrs.)	Urine flow (cc./min.)			
F.P.	30	None		None	1½	—
E.C.	18	None		None	1	—
S.S.	34	None		2	6	—
L.W.	113	None		5	3	1.006
V.E.	47	None		8	4	1.005
A.G.	180	None		6	5	1.006
R.H.	120	None		5	4	1.007
E.B.	30*	None		3	3	1.006
M.T.	128	None		8	4	1.006
M.C.	140*	6	5	None	1	1.021
D.W.	140*	12	7	None	1½	1.016
E.N.	200	20	—	20	6	—
H.G.	70*	6	5	1	6	1.006
W.M.	50*	6	5	1	5	1.006
R.F.	30*	10	7	1	4	—
F.H.	40*	8	4	1	3	1.007
A.W.	30*	16	4	1	4	1.006
J.N.	80	6	7	1	5	1.009
J.G.	25	4	10	1	4	—
N.C.	45	11	5	1	4	—
P.Y.	82	20	—	1	3	—
M.P.	157	6	6	8	3	1.004
B.B.	14	20	8	1	6	1.012
H.M.	60*	8	5	12	3	—
S.H.	73	—	—	4	4	—
E.Bl.	14	4	10	1	3	—

* Alive.

represent our total experience with hypophysectomy through November 1, 1954, Four patients have been excluded from this presentation; 2 because of immediate operative death, and 2 because of severe disease of the kidneys and ureters.

The category "immediate polyuria" in the tables deserves some discussion. Oliguria in the period following major surgery is a usual occurrence and, in fact, it has recently been hypothesized (2) that this is an expression of liberation of excess antidiuretic hormone (ADH). For purposes of classification, we have arbitarily designated urine flow above 3 cc. per minute within the first twenty-four hours as immediate polyuria. Nevertheless, the

TABLE 3. INCOMPLETE HYPOPHYSECTOMIES (8 PATIENTS)

Pt.	Survival postop. (days)	Immediate polyuria		Permanent polyuria; onset postop. (days)	Water exchange. No. of times preop. amt.	Max. Sp. Gr. postop.
		Onset postop. (hrs.)	Urine flow (cc./min.)			
G.P.	127	None	—	2	4	1.005
B.S.	212	None	—	None	2	—
S.C.	170*	None	—	2	4	1.006
M.R.	70*	None	—	None	1	—
J.V.	52	6		1	10	1.002
M.D.	85*	None		None	1	—
M.Das.	103	20	6	11	4	—
S.P.	3	20	4	1	4	—

* Alive.

patients who did not have immediate polyuria had urine outputs between 1,500 cc. and 3,000 cc. in this period. Thus, by the yardstick of non-pituitary surgery, all of our patients had relative polyuria postoperatively.

It is of practical importance that the polyuria can develop with great rapidity, the urine flow changing from 1 cc. per minute to 5 to 10 cc. per minute in one hour's time. It can begin at any time within the first twenty-four hours following operation. The variation in time of onset may be due to a number of factors, such as the degree of operative trauma, gradations in the responsiveness of the kidney to circulating ADH, or individual variations in the release of ADH.

In 3 patients there was transient polyuria followed by a permanently normal water exchange. We were unable, in these cases, to state definitely the duration of the immediate polyuria. This was due to the exigencies of the

TABLE 4. HYPOPHYSECTOMIES NOT EVALUATED FOR COMPLETENESS (5 PATIENTS)

Pt.	Survival postop. (days)	Immediate polyuria		Permanent polyuria; onset postop. (days)	Water exchange. No. of times preop. amt.
		Onset postop. (hrs.)	Urine flow (cc./min.)		
H.B.	37	None	—	2	4
B.M.	7	None	—	None	1
M.L.	56	10	6	1	3
B.F.	10	9	9	1	5
K.R.	24	2	7	1	10

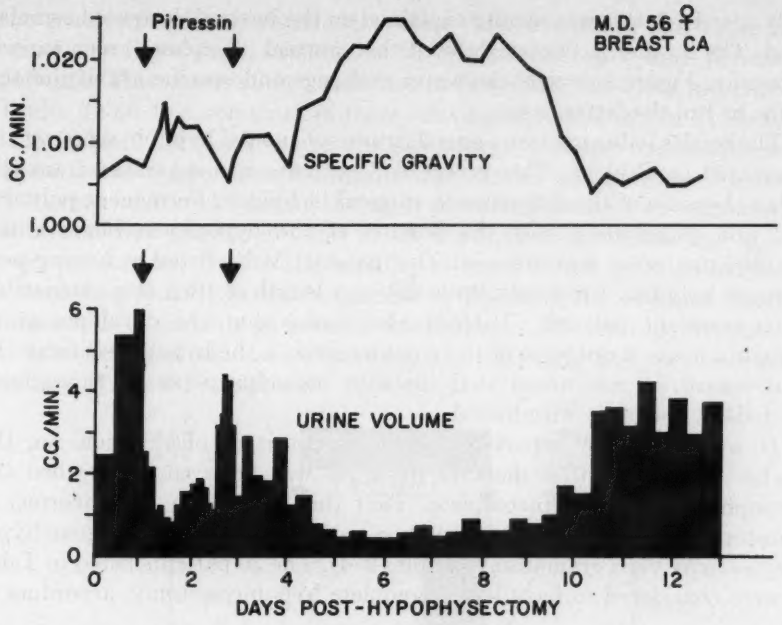

FIG. 1. Water exchange and specific gravity of urine in a patient exhibiting a "normal interphase" following hypophysectomy.

clinical situation which demanded the use of Pitressin tannate soon after polyuria became manifest. However, taking this use of Pitressin post-operatively into consideration, it is estimated that the period of transient polyuria may range from a few hours to three days.

In 4 of the patients there was a period of normal urine flow which followed the immediate polyuria, and preceded the transition to permanent

TABLE 5. EVALUATION OF COMPLETENESS OF 34 HYPOPHYSECTOMIES, EXCLUDING THE TRANS-SPHENOIDAL OPERATIONS

PERMANENT POLYURIA:	27 Present				7 Absent			
IMMEDIATE POLYURIA:	18 Present		9 Absent		2 Present		5 Absent	
HYPOPHYSECTOMY:	15 Complete	3 Incomplete	7 Complete	2 Incomplete	2 Complete	0 Incomplete	2 Complete	3 Incomplete

polyuria. In 2, this was readily explained on the basis of decreased osmolar load. The 2 other patients exhibited the "normal interphase" seen experimentally. Figure 1 depicts the water exchange and specific gravity of the urine in 1 of the latter cases.

The results following trans-antral, trans-sphenoidal hypophysectomy are presented in Table 1. This group of 8 patients was separated from the others because of the difference in surgical technique. Permanent polyuria did not occur when only the portion of the hypophysis beneath the diaphragma sellae was removed. One patient, M.F., listed as having permanent polyuria, lived only three days—a length of time also compatible with transient polyuria. Table 1 also shows that the development of polyuria is not a criterion of the completeness of the hypophysectomy. In this regard, it was noted that diabetes insipidus persisted throughout periods of cortisone withdrawal.

It was previously reported (3), on the basis of observations on the earlier operations, that diabetes insipidus was more common when the hypophysectomy was incomplete. That this impression was incorrect is substantiated by the data obtained on patients who had undergone hypophysectomy via a craniotomy (Tables 2–4). The 26 patients listed in Table 2 were considered to have had a complete hypophysectomy, according to

TABLE 6. RESULTS OF HYPERTONIC SALINE TESTS

Pt.	H_2O exchange No. of times preop. amt.	Direction of rate change in urine flow during H. saline	Rate of urine flow (cc./min.)		Evaluation of test*
			Avg. control level	Lowest level after H. saline	
B.S.	2	↑	5.2	3.2	A
F.H.	3	↑	6.5	5.7	A
S.C.	4	↑	6.2	9.4	A
S.S.	1	↑	7.9	5.0	A
R.F.	4	↑	7.1	9.0	A
P.Y.	3	↑	6.1	3.1	A
A.G.	5	↑	7.2	8.0	A
E.Bo.	3	—	6.6	4.1	A
W.M.	5	↑	6.0	3.1	A
H.G.	6	↑	7.1	6.8	A
N.C.	4	—	9.9	9.1	A
J.N.	5	↑	9.8	3.0	N
M.T.	4	↑	16.4	3.6	N
E.N.	6	↑	6.1	1.0	N
D.W.	1½	↑	7.9	1.1	N

* A = Abnormal response. N = Normal response.

the criteria previously listed. It is clear from this table that there is no correlation between completeness of hypophysectomy and permanent polyuria. This observation seems also to be valid in the data presented in Tables 3 and 4. A summary of these relationships is shown in Table 5.

In Table 6 are listed the results of intravenous hypertonic saline tests. These tests were performed as outlined by Carter and Robbins (1), except that water restriction during the night preceding the test was minimal; results were not considered valid unless the control urine flow was at least 5 cc. per minute. Due to the inability of many of our patients to excrete a water load quickly, it was impossible to determine the response to hypertonic saline in as many patients as was desirable. The average control level of urine flow, and the lowest value reached following the hypertonic saline test are also listed in Table 6.

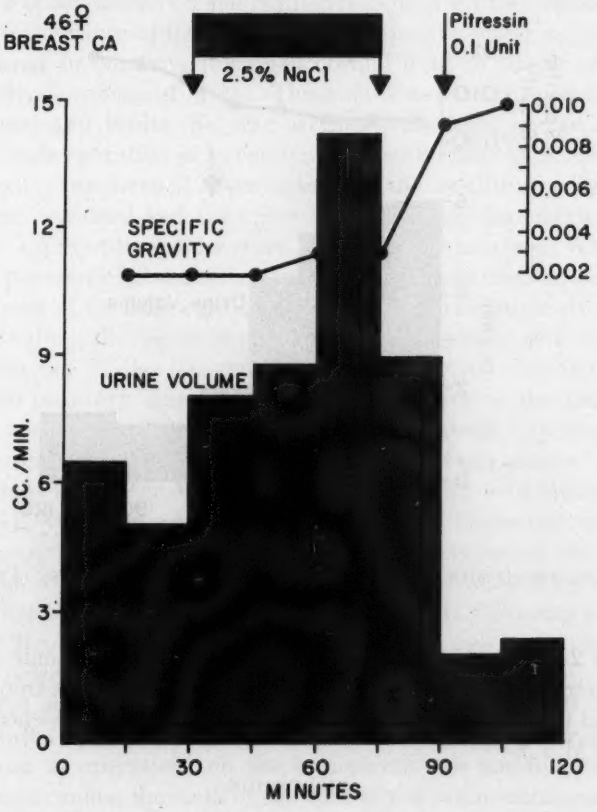

FIG. 2. Abnormal results with Carter-Robbins hypertonic saline test, after hypophysectomy (Patient F. H.). Daily urinary volume, 5,000 cc.

Although these patients generally did not attain levels of daily water exchange as high as those seen in diabetes insipidus, their responses to hypertonic saline were usually grossly abnormal. This response, characteristic of diabetes insipidus, was noted even in Patients B.S. and S.S., whose daily water exchange was close to normal. Three patients, J.N., M.T. and E.N., had a normal response to the hypertonic saline test, although their daily polyuria was definite and marked.

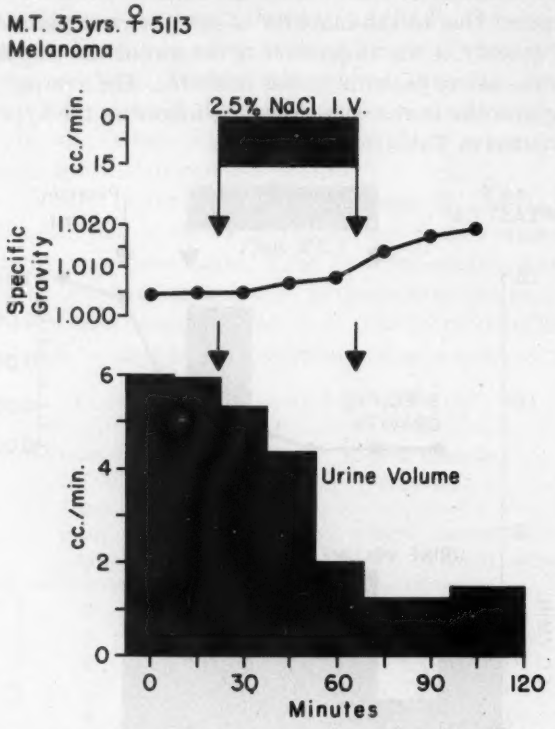

FIG. 3. Normal results with Carter-Robbins test after hypophysectomy (Patient M. T.). Daily urinary volume, 8,000 cc.

Figures 2 and 3 show representative results of hypertonic saline tests which illustrate respectively the abnormal type of response frequently seen (F.H.) and the normal response that may occur in the presence of marked polyuria (M.T.).

DISCUSSION

Following the original observations of Frank (4) in 1910, that the posterior pituitary gland is related to diabetes insipidus, the elucidation

of the pathophysiology of the disease became increasingly difficult. This was due to the profusion of lesions in the vicinity of the hypophysis and hypothalamus, and the difficulty in localizing precisely the effects of these lesions. Furthermore, the neuroanatomy of the hypothalamic-hypophysial system was not appreciated, although Cajal (5) had described the nerve fibers from the hypothalamus to the pars nervosa twenty years earlier.

The experimental approach to the pathogenesis of diabetes insipidus became increasingly fruitful in the 1930's, and several groups of investigators were able to delineate its features. Fisher, Ingram and Ransom (6) summarized a series of experiments in the cat and monkey that clearly demonstrated the dependence of the pars nervosa on the supra-optic nuclei of the hypothalamus. Subsequently (7) they were able to make quantitative observations on the relationship between the number of cells remaining in the supra-optic nuclei and variations in water exchange. The water exchange in monkeys remained normal if 12 to 16 per cent of the neurohypophysis remained intact. These data were confirmed in the dog by Heinbecker and White (8), who sectioned the stalk at various levels, either as a single operation or in combination with removal of the posterior lobe or median eminence. After assessing the resulting polyuria, the animals were sacrificed and the effects of retrograde degeneration on the cells of the supra-optic nuclei were measured at autopsy. When fewer than 5 to 7 per cent of these cells remained, there was marked polyuria. If over 15 per cent of the cells were intact, there was no polyuria. At stages between these values, the degree of polyuria varied inversely with the number of cells remaining. Keller has repeatedly noted (9, 10) that following removal of the pituitary gland and stalk, the severity of the polyuria depends upon the degree of damage to the ventral anterior hypothalamus.

Confirmation of these results in the human has been scanty. A patient with malignant hypertension had section of the stalk without subsequent polyuria (11). At autopsy five months later, about 15 per cent of the cells of the supra-optic nuclei were intact. Dandy (12) reported the case of a young woman who had section of the pituitary stalk, followed by a polyuria of 3,000–4,000 cc. per day. Another case of polyuria following partial destruction of the supra-optic nuclei by an angioma was noted in a review (13) of the pathology of diabetes insipidus.

The clinical observations of neurosurgeons were summarized by Dandy (12), who stated that 1) tumors confined to the sella never cause diabetes insipidus, and 2) operations on the hypophysis are not followed by diabetes insipidus unless the stalk or the base of the brain is traumatized.

Our results support the concept that only a small percentage of viable supra-optic cells need remain to prevent polyuria. Thus, when hypophy-

sectomy was confined to removal of tissue below the diaphragma sellae, permanent polyuria did not develop. However, following suprasellar removal of the pituitary, the occurrence of permanent polyuria was frequent but unpredictable. As there was no ancillary evidence of hypothalamic damage, it is probable that partial removal of the stalk with the hypophysis reduced the number of functioning cells of the supra-optic pars nervosa system to a level below that compatible with normal fluid exchange in the patients with polyuria. It is not implied that variations in the degree of polyuria depend solely on the level of stalk section. One other important factor is the daily osmolar load.

The appearance of immediate polyuria following surgery, with return to normal levels of urine flow subsequently, is not completely explained by available experimental data. It has been assumed (6, 14) that this transient polyuria is due to operative damage to the cells producing ADH, resulting in a temporarily decreased release of the hormone. Recently (15) the renal concentrating ability has been tested in dogs during this phase, following transection of the pituitary stalk and damage to the hypothalamus. Four of 9 dogs were able to handle an osmotic load normally. This suggests that ADH may be released during the transient phase in response to an osmotic load in some individuals.

The parallel between animal experiments and human hypophysectomies may be further illustrated by consideration of Figure 1. The patient, whose water exchange is represented in this chart was the first to have a hypophysectomy by the transtemporal route. At autopsy, it was found that the pituitary stalk had been removed and the sellar contents were intact. Following surgery she demonstrated the triphasic response described by Fisher *et al.* (16) and O'Connor (17) following interruption of the supra-optic pars nervosa system, and by Heinbecker and White (8) following high stalk section. The immediate polyuric phase has already been considered. The interphase of normal urine flow was considered to be due to release of preformed ADH. The rise in specific gravity during this period made this the most likely explanation and indicated that a decrease in osmolar load was not the cause of the decreased urine flow. The next phase of polyuria was permanent at a level of 3,000–4,000 cc. per day. This is the only case reported in man of a triphasic response following section of the stalk. The great similarity of these changes to those seen experimentally further strengthens our belief that the conclusions arrived at in animals are valid in man.

It has been stated by Beaser (18) and Talbot *et al.* (19) that ADH has an all-or-none effect; so that if any ADH is present, there is no polyuria. The latter authors admit, however, that it is difficult to explain the wide variations in the degree of polyuria seen in patients with diabetes insipidus.

The over-all correspondence of our results with those obtained by Heinbecker and White (8) favors the concept that below a certain critical amount of ADH, the urine flow varies with the amount of ADH produced. This is illustrated in Figure 4, taken from Heinbecker's paper; the relatively low degree of polyuria would then correspond to the points on the abscissa between 5 and 15 per cent Although no studies were made of osmotic load, most of these patients during the period of study were eating an amount of food comparable to that during the preoperative period.

The responses to the hypertonic saline test are of physiologic interest.

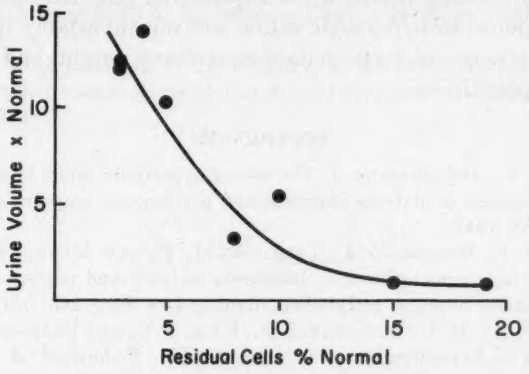

FIG. 4. Relationship between water exchange and the number of cells in the supraoptic nuclei. (Reproduced by permission of the publishers of the American Journal of Physiology; Figure 3 from Heinbecker, P., and White, H. L.: *Am. J. Physiol.* **133**: 582. 1944 (ref. 8).

If we can assume that patients showing permanent polyuria still have some degree of function of the hypothalamic-pars nervosa system, then a response to hypertonic saline could be expected. This, in fact, did occur in 3 patients, indicating that the small remnant of functioning pars nervosa is capable of releasing extra ADH in response to extreme hypertonicity.

These normal results in 3 patients with polyuria would suggest that the findings with the hypertonic saline test should be cautiously interpreted when used as the sole criterion for differentiating between diabetes insipidus and psychogenic polydipsia. That the distinction between these states may not be quite so clear-cut is suggested by two groups of workers (15, 20) who have adduced evidence that dogs with diabetes insipidus may have disturbances both in the thirst mechanism and in renal concentrating ability. Thus, conceivably, an intermediate state can exist, in which there is a deficiency of ADH, as well as polydipsia.

SUMMARY

1. Variations in water exchange following hypophysectomy in man are reported.

2. The presence or absence of polyuria cannot be used as a criterion for determining the completeness of hypophysectomy.

3. The occasional development of immediate polyuria, without subsequent permanent polyuria, is attributed to temporary "shock" of the remaining pars nervosa. This and the triphasic response noted following section of the stalk parallel experimental findings in animals.

4. It is suggested that the degree of polyuria depends in part upon the amount of functioning tissue in the supra-optic pars nervosa system.

5. The response to hypertonic saline will not invariably distinguish between the polyuria of hypothalamic-pituitary origin and that due to psychogenic polydipsia.

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RELATIONSHIP OF DIURNAL VARIATION IN 17-HYDROXYCORTICOSTEROID LEVELS IN BLOOD AND URINE TO EOSINOPHILS AND ELECTROLYTE EXCRETION*

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THE state of sleep is associated with many measurable changes, including an increased number of mitoses (1, 2), a high eosinophil count (3, 4) increased liver glycogen (5, 6), decreased blood epinephrine (7), decreased excretion of water (8, 9), electrolytes (8, 10), uric acid (11) and 17-ketosteroids (12), and lowered body temperature (13). The urinary excretions mentioned decrease in spite of unchanged renal function (14). Then in apparent preparation for the day's activity, a rapid reversal of these functions occurs, a few hours before awakening. It is evident that the rhythm of activity of many of these substances may actually be caused by a change in adrenal gland activity.

A diurnal variation in the eosinophil count has been shown by Halberg (15) and Rud (4), with the maximum drop occurring between 6:30 and 9:30 A.M. Diurnal variation in the level of plasma 17-hydroxycorticoids between 8:00 A.M. and 12:00 midnight has been shown by Bliss *et al.* (16). It has been demonstrated by many investigators that the excretion of sodium and potassium is much less by night than by day, even if electrolyte intake is equally distributed at short intervals throughout the twenty-four hours.

The object of this investigation was to determine whether there is a diurnal variation in 17-hydroxycorticosteroids in plasma and urine, and to examine any relationship between this rhythm and that of eosinophil activity and electrolyte excretion. Any possible interrelationship might become more obvious in this way. An abstract of these studies was reported earlier (17).

METHODS

The 25 subjects studied were adult males in apparently normal health as determined by routine examination. They were served a general hospital diet and allowed to be am-

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bulatory. Urine was collected on ice without preservative at six-hour intervals beginning at midnight; it was frozen until determinations were made. Blood specimens were obtained at 6:00, 9:00, 15:00 (3 P.M.) and 21:00 (9:00 P.M.). The plasma was separated immediately and frozen. The times of withdrawal of blood were selected with the hope that the subjects would be disturbed as little as possible during the night. Electrolyte excretion studies were carried out with 4 physicians as subjects and this group ate two graham crackers and 100 ml. of milk every three hours for a 27-hour period. The diet was begun three hours before the first urine collection was started.

Plasma 17-hydroxycorticosteroid determinations were made, using the method of Nelson and Samuels (18). Urinary 17-hydroxycorticosteroid excretion was measured by the method of Reddy *et al.* (19). Circulating eosinophils were stained by a modification

TABLE 1. URINARY 17-OH-CS VALUES IN 22 SUBJECTS. (VALUES ARE RELATIVE, IN TERMS OF PER CENT OF INDIVIDUAL'S MEANS)

	Mean (mg./hr.)	Per cent of mean (6-hour periods)			
		0–6:00 A.M.	6:00–12:00	12:00–18:00	18:00–24:00
1. F.R.	.31	76	108	118	100
2. M.K.	.24	58	121	112	112
3. E.F.	.54	40	123	148	87
4. M.T.	.42	7	292	95	5
5. W.L.	.41	47	244	107	2
6. F.S.	.10	42	213	109	35
7. L.B.B.	.55	116	136	101	47
8. R.D.	.34	42	141	147	67
9. W.C.	.23	66	206	110	19
10. D.O.	.34	41	62	231	67
11. L.B.	.17	47	210	109	34
12. R.K.	.33	39	101	115	149
13. R.D.	.64	39	122	155	83
14. J.P.	.43	51	130	148	72
15. J.J.	.51	132	109	104	51
16. A.G.	.18	68	158	73	102
17. F.G.	.20	47	104	122	125
18. F.K.	.17	10	201	78	109
19. L.S.	.43	78	154	130	37
20. F.S.S.	.26	15	141	81	161
21. W.F.	.36	78	161	99	62
22. T.C.	.51	49	164	122	66
Mean	.348	54%*	154%*†	122%	72%†
S.D.	.146	30	54	33	43
Mean (mg./hr.)		.188	.527	.435	.224

* Statistical analysis between these means, $T = 7.6$; $P = < .01$.

† Statistical analysis between these means, $T = 5.6$; $P = < .01$.

of the method of Randolph (20). Concentrations of sodium and potassium in the urine were determined by the use of a Baird internal standard flame photometer.

RESULTS

A diurnal variation in plasma and urinary 17-hydroxycorticosteroid (17-OH-CS) levels was found. This was associated with a diurnal variation in the number of eosinophils. Tables 1–3 show the mean values obtained in 25 subjects and the percentile variation from the mean for eosinophils and

TABLE 2. PLASMA 17-OH-CS VALUES IN 13 SUBJECTS. (VALUES ARE RELATIVE, IN TERMS OF PER CENT OF INDIVIDUAL'S MEAN)

	Mean (γ/100 cc.)	Per cent of mean			
		6:00 A.M.	9:00	15:00	21:00
1. F.R.	7.7	192	82	52	76
2. M.K.	11.75	42	194	148	10
3. E.F.	16.1	115	119	121	44
4. M.T.	18.4	81	116	117	85
5. W.L.	8.3	78	163	93	65
6. F.S.	6.4	132	158	65	45
7. V.S.	7.8	156	93	51	98
8. F.B.	13.1	180	80	136	4
9. W.C.	5.3	240	118	19	22
10. D.O.	12.2	126	124	50	
11. L.B.	7.6	79	179	41	
12. R.K.	4.2	142	29	166	61
13. G.T.	12.4	80	142		78
Mean	10.1	126%*	123%†	88%	54%*†
S.D.	4.3	±56	45	48	31
Mean γ/100 cc.		11.0	12.7	9.4	5.5

* Statistical analysis between these means, T = 3.8; P = <.01.
† Statistical analysis between these means, T = 4.29; P = <.01.

plasma and urinary 17-OH-CS. One group of 7 males was studied and complete values for plasma, urinary 17-OH-CS and eosinophils were obtained (Fig. 1). The urinary excretion from 0 to 6:00 A.M. was very low but rose to a sharp peak from 6:00–12:00 A.M. and dropped off slowly thereafter. Plasma 17-HO-CS concentrations rose to a peak at 9:00 A.M. and dropped to a low point at 9:00 P.M. The blood eosinophil level rose and fell in inverse relationship to the plasma 17-OH-CS levels. The values are reported in terms of per cent of the mean, in order to equalize varia-

tion in subjects with varying baselines of excretion. A larger group of subjects was studied, with only individual variables being obtained in some of them (Fig. 2). The values corresponded closely to those of the smaller group, except that the plasma 17-OH-CS concentration was higher at 6:00 A.M. than at 9:00 A.M. Statistical analysis of some of the more

TABLE 3. EOSINOPHILS IN 17 SUBJECTS. (VALUES ARE RELATIVE, IN TERMS OF PER CENT OF INDIVIDUAL'S MEAN)

	Mean No./cu. mm.	Per cent of mean			
		6:00 A.M.	9:00	15:00	21:00
1. F.R.	156	117	74	99	110
2. M.K.	193	129	89	69	112
3. E.F.	49	178	96	35	90
4. M.T.	231	136	69	81	114
5. W.L.	198	103	92	108	98
6. F.S.	133	108	81	122	87
7. V.S.	393	169	62	86	83
8. F.B.	402	143	94	49	112
9. W.C.	202	109	87	106	98
10. D.O.	159	59	108	126	108
11. L.B.	238	88	55	104	152
12. R.F.	148	93	97	71	138
13. F.S.	240	95	84	106	98
14. J.P.	144	104	70	87	138
15. J.J.	132	79	79	63	180
16. A.G.	531	66	89	135	112
17. F.G.	341	117	81	100	101
Mean	229	111%*	82%*†	91%	115%†
S.D.	122	32	14	27	25

* Statistical analysis between these means, T = 3.4; P = <.01.
† Statistical analysis between these means, T = 4.8; P = <.01.

marked variations showed them to be highly significant statistically, as noted in Tables 1–3. It should be emphasized that the most consistent and most marked variation was obtained in the urinary 17-OH-CS excretion. In only 1 instance out of 22 was the excretion from 0–6:00 A.M. greater than from 6:00–12:00 noon.

Another group of subjects was studied while having a constant food intake every three hours; they were allowed normal activity. A marked rhythm was noted in the eosinophil counts and in urinary 17-OH-CS.

sodium, and potassium excretion (Fig. 3). The rise in urinary 17-OH-CS began in the three-hour period before the rise in urinary sodium and potassium. Examination of the data for each subject (Table 4) revealed that the peak corticoid excretion usually occurred in the three-hour period before the peak for potassium; less frequently the peaks occurred in the same period. Again, eosinophil rhythm was the reverse of that of plasma 17 OH-CS.

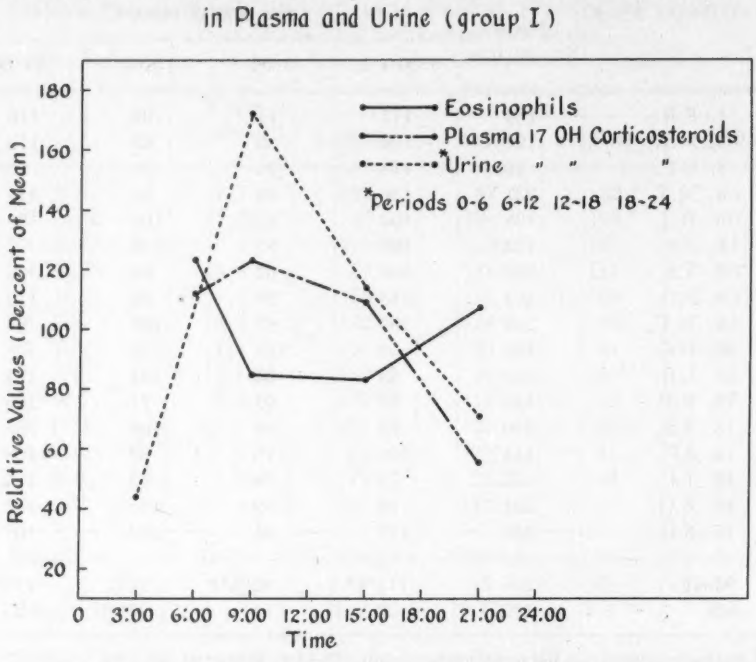

FIG. 1. Diurnal variations in 7 normal subjects. The collections were made simultaneously.

* Time periods, starting with 0–6 A.M.

A single male subject was studied for all individual variables and the results are shown in Figure 4. Plasma 17-OH-CS levels began to rise at 3:00 A.M., as the eosinophils began to fall. At 6:00 A.M. urinary 17-OH-CS excretion began to rise, and at 9:00 A.M. urinary potassium began to rise. Sodium excretion was not well correlated with any other variable in this subject. On the other hand, the urinary excretion of potassium was closely correlated with that of 17-OH-CS.

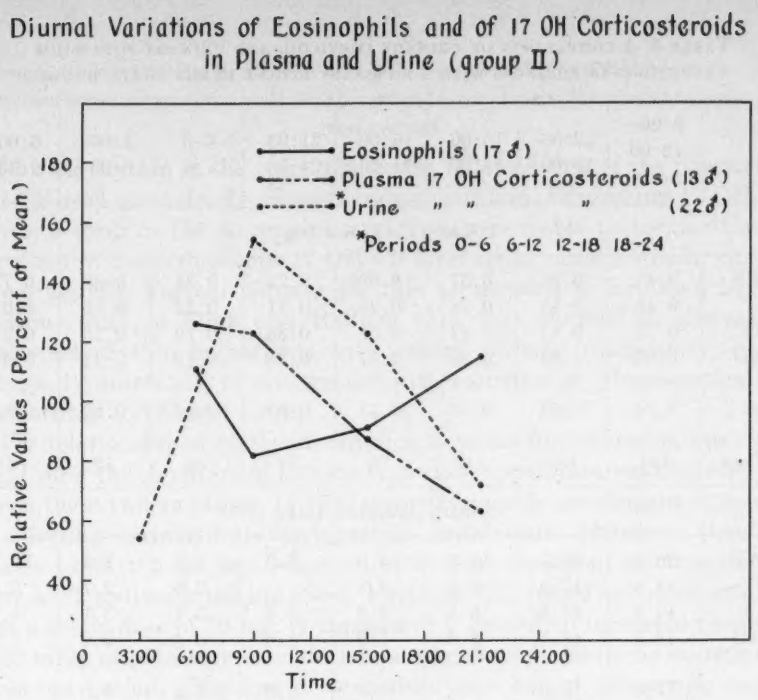

FIG. 2. Pooled data for diurnal variation in individual variables.
* Time periods, starting with 0–6 A.M.

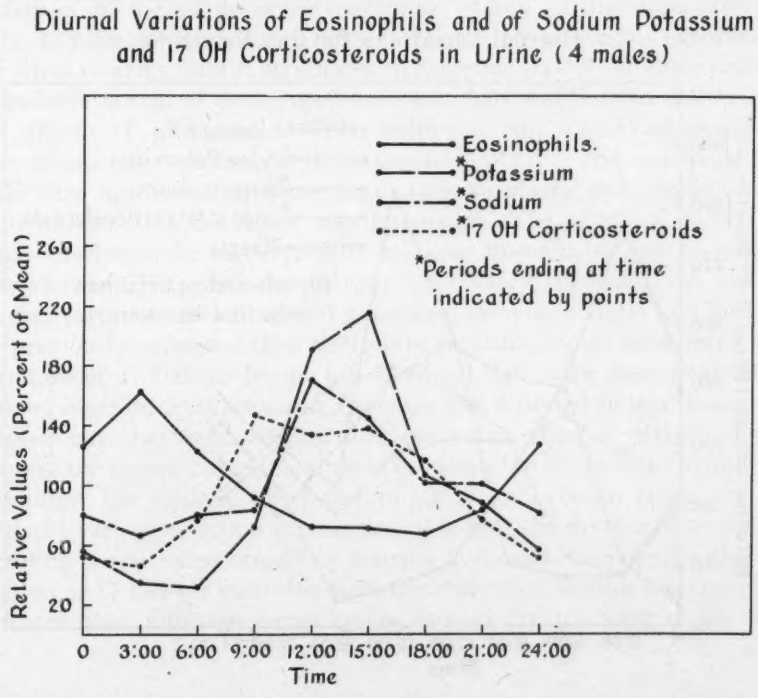

FIG. 3. Diurnal variations in 4 normal males, with a constant intake every three hours.

TABLE 4. A COMPARISON OF URINARY CORTICOID AND URINARY POTASSIUM EXCRETIONS IN SUBJECTS WITH A CONSTANT INTAKE EVERY THREE HOURS

	9:00–12:00 NOON	12:00–15:00	15:00–18:00	18:00–21:00	21:00–24:00	0–3 A.M.	3:00–6:00	6:00–9:00
				Urinary Corticoids (mg.)				
1. WF	0.44	0.36	0.37	0.095	0.22	0.21	0.38	0.77
2. JV	0.46	0.55	0.28	0.29	0.11	0.23	0.45	0.69
3. EF	0.75	0.82	0.77	0.58	0.36	0.19	0.25	0.57
4. RD	0.99	1.03	0.94	0.67	0.38	0.19	0.32	0.57
Mean	*0.66*	*0.69*	*0.59*	*0.41*	*0.27*	*0.21*	*0.35*	*0.65*
% of Mean	*134*	*139*	*116*	*79*	*54*	*46*	*79*	*147*
				Urinary potassium (mEq.)				
1. WF	20.3	18.3	13.8	6.9	5.3	2.3	3.3	11.7
2. JV	18.8	17.9	9.2	8.8	—	3.0	2.7	5.8
3. EF	15.8	23.6	6.5	8.8	5.7	3.1	3.1	3.7
4. RD	14.4	18.7	8.9	7.2	4.0	3.8	2.9	5.6
Mean	*17.3*	*19.6*	*9.6*	*7.9*	*5.0*	*3.1*	*3.0*	*6.7*
% of Mean	*190*	*217*	*105*	*88*	*56*	*35*	*33*	*72*

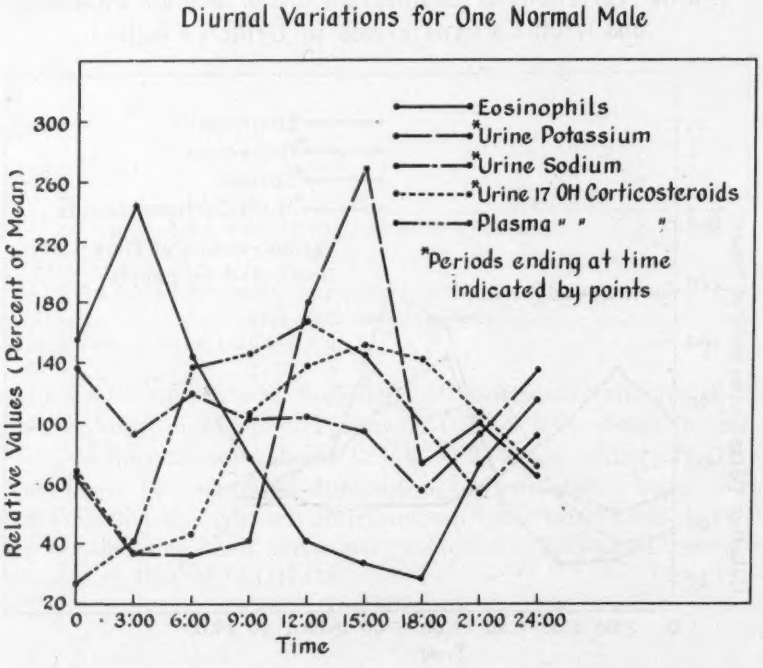

FIG. 4. Diurnal variations for a normal male, with a constant intake every three hours.

DISCUSSION

Diurnal rhythm in the eosinophil count followed that of the plasma 17-OH-CS level quite closely. Since the administration of exogenous 17-OH-CS causes a drop in the eosinophil level, it is reasonable to assume that a variation in the endogenous 17-OH-CS level could cause a similar change in eosinophils. Further support for this assumption is furnished by the previous work of Flink and Halberg (21), who showed an absence of eosinophil rhythm in patients with severe adrenal insufficiency, either previously untreated, or not treated with cortisone or adrenocortical extract within forty-eight hours.

The relationship of corticoid variation to potassium excretion was striking. Under the condition of this study, a close correlation was observed between these two variables. 11,17-Oxycorticosteroids are thought of mainly as affecting carbohydrate and protein metabolism. However, they are known to have a marked effect on potassium excretion, as compared to their weak sodium-retaining effect. Fourman (22) found in one experiment that a single dose of 50 mg. of compound F caused an increased excretion of 20 mEq. of potassium in eight hours, when compared to the excretion in a control period. This loss of potassium was out of proportion to the expected loss from protoplasmic breakdown, as estimated from simultaneous nitrogen excretion. In another subject (23) he found that a single injection of 30 mg. of desoxycorticosterone caused an increased excretion of 11–12 mEq. of potassium in forty-two hours. In these acute experiments the effect of compound F on potassium excretion was of the same order of magnitude as that of desoxycorticosterone. Luft and Sjogren (24) studied the effects of prolonged ACTH, cortisone, and desoxycorticosterone administration on patients with rheumatoid arthritis and concluded that a division into salt and water regulating hormones and carbohydrate-protein regulating hormones was impossible. The effect of ACTH on potassium excretion was especially marked. Rosenbaum and co-workers (25) showed that cortisone therapy completely disrupted the normal diurnal variation in sodium and potassium excretion. Borst and DeVries (9) previously suggested that electrolyte excretion varies secondarily to a variation of 17-OH-CS levels, but they did not make measurements of adrenal function. Stanbury and Thomson (26) objected to this theory and pointed out that both sodium and potassium show a matutinal rise, whereas the known adrenal hormones influence the electrolytes in opposite directions. The striking correlation in our study between potassium and corticoid variations makes it seem probable that the rhythm of potassium excretion is controlled largely by adrenal hormones. Conversely, the correlation of 17-OH-CS excretion with the individual sodium excretion pattern was poor, although group figures showed them to vary in the same

direction. It would appear that there is only a small lag between the maximum 17-OH-CS level in the plasma and the appearance of 17-OH-CS in the urine. Three hours after that, the maximal amounts of electrolytes are excreted.

It should be noted that administered 17-OH-CS can actually increase both sodium and potassium excretion under special circumstances (27–29), especially at the dosage level concerned in the endogenous production of 17-OH-CS (Laidlaw (30)). This would indicate that the adrenal cortex causes the diurnal variation in both electrolytes. However, the poor correlation between sodium and 17-OH-CS excretion in 1 subject in this study would tend to indicate that these corticosteroids do not always have a predominant role in regulating sodium rhythm.

Activity and assumption of the upright posture are not necessary for the early morning increase in plasma and urinary 17-OH-CS levels or for the drop in eosinophils. These changes have been shown to begin when the individual is still asleep; they reach a higher level when morning activity would be expected to begin.

CONCLUSION

1. A definite diurnal variation was observed in the eosinophil count, in the level of plasma 17-hydroxycorticoids, and in urinary 17-hydroxycorticoid, sodium and potassium excretions.

2. Variation in eosinophils was closely related to variations in plasma 17-hydroxycorticoid levels.

3. In the urine, potassium excretion was closely correlated with 17-hydroxycorticosteroid excretion.

4. Urinary sodium excretion was not closely correlated in individual subjects with any of the variables measured, although it followed the same trend as potassium excretion when group figures were used.

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ACTH RESISTANCE IN ACROMEGALY

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EOSINOPENIC response has been criticized clinically and experimentally since its widespread adoption as a criterion for evaluation of adrenocortical function (1–4). Investigating the eosinopenic response in neuroendocrine disorders, we found either resistance or a paradoxical reaction to subcutaneous insulin, epinephrine, and intramuscular ACTH in acromegalic patients (5). These findings could not be solely explained by the concept of diminished adrenocortical reserve, in view of the generally accepted consideration that there is hyperplasia of the adrenal cortex in acromegaly (6). We then attempted to test the same subjects by intravenous infusion of ACTH and found that the circulating eosinophils responded normally in almost all cases. Therefore, the phenomenon observed following intramuscular injection may be due to local inactivation of ACTH and designated properly as "ACTH resistance." This term originated with Forsham *et al.* (7, 8), who found that repeated intramuscular administration caused increasing unresponsiveness to the hormone; nevertheless, ACTH-resistant subjects were found to respond normally to intravenous administration of the same material. They postulated that the cause of this phenomenon might be the inactivation of the hormone at the site of injection. It was with particular interest, therefore, that we observed the same lack of response in a series of untreated acromegalics. Furthermore, responsiveness to ACTH was improved following the administration of testosterone propionate in one of the cases.

We should like to suggest that these facts are related intimately to the patho-physiologic status of acromegaly, and may shed light on one of the mechanisms of "ACTH resistance."

MATERIALS AND METHODS

Six patients (3 male and 3 female) with typical signs of acromegaly were studied (Table 1). In addition to the signs of acral hypertrophy, Patients SGY and SSK showed marked bitemporal hemianopsia, and Patient KID had a slight derangement of the visual field. Glycosuria was present in 2 cases (KNK and TKY); albuminuria was present in 1 (KID), and the Wassermann reaction was positive in 1 (TKY). Three patients

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(SGY, TKY and KID) had exophthalmos, and KNK, in particular, complained of persistent and severe headache. Slightly high blood pressures were observed in 2 instances (SGY and KID). In all cases the roentgenograms showed marked ballooning of the sella turcica and characteristic tufting and thickness of the terminal phalanges. Osteoporosis was also observed in all cases, especially in the knee joint.

These subjects were admitted to Nagoya University Hospital and were investigated

TABLE 1. PROTOCOLS

Patient	SYG	KID	KNK	TNG	TKY	SSK
Age & Sex	28 M	32 M	49 M	29 F	41 F	54 F
Height	182 cm.	158 cm.	165 cm.	143 cm.	164 cm.	
Girth of chest	110 cm.	100 cm.	92 cm.	90 cm.	92 cm.	
Weight	99 Kg.	72 Kg.	66 Kg.	55 Kg.	69 Kg.	
Diagnosis	Gigantism; acromegaly	Acromegaly	Acromegaly	Acromegaly	Acromegaly	Acromegaly
Age at onset	16 yr.; incr. in ht.	24 yr.; visual disturb. lt. eye	38 yrs.; persistent headache	25 yr.; irreg. menstr.	28 yr.; enlargement of hands & feet	
Pertinent history & physical findings	Excessive ht. & wt.; acral hypertrophy & prognathism; bitemp. hemianopsia	Excessive wt.; acral hypertrophy & prognathism	Acral hypertrophy; dermolysis & lipoma on back	Acral hypertrophy; exostosis & prognathism	Persistent headache; acral hypertrophy & prognathism	Acral hypertrophy & bitemp. hemianopsia
Headache	(−)	(−)	(++)	(−)	(+)	(−)
Subjective visual disturbances	(++)	(+)	(−)	(−)	(−)	(++)
Mental activity	Good	Good	Good	Good	Good	Good
Libido & potentia	(±)	(−)	(−)	(−)	(−)	(−)
Arthralgia	(+)	(+)	(+)	(±)	(+)	(−)
Exophthalmos	(+)	(+)	(−)	(−)	(+)	(−)
Blood pressure	140/60	142/92	128/75	110/70	130/80	
Urinalysis						
Albumin	(−)	(++)	(−)	(−)	(−)	(−)
Sugar	(−)	(−)	(++)	(−)	(+)	(−)
X-ray exam.						
Sella	Marked ballooning	Marked ballooning	Marked ballooning	Marked ballooning	Marked ballooning	Marked ballooning
Epiphyses	Closed	Closed	Closed	Closed	Closed	Closed
Spine & hand	Typical for acrom.	Typical for acrom.	Typical for acrom.	Typical for acrom.	Typical for acrom.	Typical for acrom.
Osteoporosis	(+)	(+)	(+)	(+)	(+)	(+)
Visual field encroachment	Remarkable	Slight	None	None	None	Remarkable
B.M.R. (%)	−3	−20	−14	—	−34	—
Wassermann	(−)	(−)	(−)	(−)	(+)	(−)

for responsiveness to ACTH, administered by both intramuscular and intravenous routes. The Robinson-Power-Kepler water test (R.P.K. test) (9) was carried out, and urinary 17-ketosteroids (17-KS) were determined (10). ACTH tests started between 9:00 and 10:00 A.M. A dose of 25 I.U. of ACTH (Armour) dissolved in 3 ml. of physiologic saline solution was given intramuscularly in all 6 cases (Thorn's method) (1). In addition, 4 of the patients received a continuous infusion of 20 I.U. of ACTH (Armour) in 500 ml. of 5 per cent dextrose solution over a period of eight hours (Renold's method) (8).

Eosinophils were counted directly, using eosinoacetone diluent and the Fuchs-Rosenthal chamber. Blood samples were obtained in duplicate from earlobes.

In 2 patients (KID and KNK) testosterone propionate (T.P.) was administered intramuscularly for treatment of the acromegaly. Then the eosinopenic responses were tested by intramuscular administration of ACTH (Armour) before, during, and after treatment. In Patient KID, the T.P. was administered in doses of 25 mg. twice a week for six weeks for a total of 450 mg. In Patient KNK, the T.P. was injected in doses of 25 mg. every other day for three weeks, and then injections were given daily, for a total of 650 mg. Blood samples were drawn from the cubital vein of the fasting subjects, and heparinized. The level of inorganic phosphorus in the plasma was determined by the method of Youngburg and Youngburg (1930). Chlorides in the urine and plasma were assayed by the methods of Volhard and Harvey (1910) and Rusznyak (1921), and the method of Natelson *et al.* (11) was applied for the determination of urea-nitrogen.

TABLE 2. EOSINOPHIL CHANGES FOLLOWING SINGLE INTRAMUSCULAR INJECTION OF 25 I.U. OF ACTH (ARMOUR)

Patient, Age, Sex			Date of exam.	Pre-injection eosinophil counts (per cu. mm.)	Changes in circulating eosinophils (%)				Remarks
					1 hr.	2 hrs.	3 hrs.	4 hrs.	
KNK	49	M	Sept. 26, '52	525	− 9	+ 4	−13	− 4	Before T.P.*
			Nov. 22, '52	384	+36	− 9	−12	−16	After T.P.
			June 10, '53	336	+ 7	+43	+27	+ 9	6.5 months after therapy
SGY	28	M	Jan. 9, '54	609	+15	+ 7	+ 5	− 3	
TKY	41	F	Oct. 3, '53	150	+18	+18	+28	− 8	
			July 17, '54	135	−11	−22	+ 6	+ 9	After antiluetic therapy†
TNG	29	F	Sept. 9, '53	274	+22	+32	+16	−17	
KID	32	M	Aug. 8, '52	160	+80	+ 9	+42	+84	Before T.P.
			Sept. 26, '52	198	−21	− 9	−13	−32	During T.P.
			Nov. 22, '52	187	−41	−25	−59	−54	After T.P.
SSK	54	F	Feb. 24, '52	1856	− 1	+42	+48	+114	

* Administration of testosterone propionate.
† Penicillin in oil, 20.4 million units.

RESULTS

As shown in Table 2, no eosinopenic response was observed in any case of non-treated acromegaly. In contrast, continuous intravenous infusion of ACTH induced a definite reduction of the circulating eosinophils in 3 of 4 cases (KNK, SGY and TKY (Table 3)). At the eighth hour of infusion the decreases were 95, 84 and 77 per cent, respectively. These data are compatible with the negative results of the R.P.K. test and almost normal urinary excretion of 17-KS (Table 4), and suggest that there was no adrenocortical insufficiency in these cases. Patient KID possibly belonged also in this category in spite of the positive R.P.K. result, which may have

TABLE 3. CONTINUOUS INTRAVENOUS INFUSION OF 20 I.U. OF ACTH (ARMOUR) OVER AN 8-HOUR PERIOD

Patient	Age	Sex	Date of exam.	Pre-infusion eosinophil counts (per cu. mm.)	Decrease in circulating eosinophils (%)			
					4 hrs.	8 hrs.	12 hrs.	24 hrs.
KNK	49	M	June 6, '53	350	−68	−95	−97	−78
SGY	28	M	Jan. 30, '54	681	−66	−84	−82	−37
TKY	41	F	Nov. 20, '53	208	−62	−77	−76	−61
TNG	29	F	Nov. 24, '53	324	−20	−26	−20	+13

been mainly attributable to renal dysfunction, as he died of uremia about two years later, on February 20, 1955. Sclerosis of the kidney and a very large hypophysis were found at autopsy, but his adrenals were somewhat hypertrophied. Eosinopenic responses following both intramuscular and intravenous ACTH were inadequate in Patient TNG, who also showed a positive result with the R.P.K. test and diminished excretion of urinary

TABLE 4. ROBINSON-POWER-KEPLER TEST AND URINARY 17-KETOSTEROIDS

Patient	Robinson-Power-Kepler test								17-KS† (mg./24 hrs.)
	Chloride (mg./100 ml.)		Urea-nitrogen (mg./100 ml.)		Vol. of urine (ml.)		Factor A*	Result	
	Plasma	Night urine	Night urine	Plasma	Largest hourly specimen	Night urine			
KNK	316	442	1,670	26.0	1,000	400	115	(−)	5.7
SGY	580	330	975	11.7	454	497	134	(−)	9.0
TKY	368	546	573	12.6	770	570	41	(−)	6.8
TNG	380	925	469	6.0	80	430	6	(+)	3.2
KID	328	1,023	975	41.0	560	800	5	(+)	10.3

* $A = \frac{\text{Fasting plasma chloride}}{\text{Urine chloride in night specimen}} \times \frac{\text{Urine urea-nitrogen in night specimen}}{\text{Plasma urea-nitrogen}} \times \frac{\text{Vol. of largest hourly day specimen}}{\text{Vol. of night specimen}}$

† Average for 3 days.

17-KS. In this case, there were no clinical signs and symptoms of Addison's disease or renal disorders, but the data suggest the possible presence of adrenocortical insufficiency. Concerning Patient SSK, unfortunately there were not enough data to justify a conclusion regarding the status of adrenocortical function.

Thus, it is suggested that in our series the absence of eosinopenic response to the intramuscular administration of ACTH, namely "ACTH resistance," was not due to adrenocortical insufficiency but rather to the inactivation of ACTH at the site of injection.

In 1 (KID) of 2 patients treated with T.P. there was a beneficial effect on both subjective and objective symptoms, *i.e.*, there was recovery from impotence, alleviation of headache, improvement of vision, and enlargement of the visual field. Plasma levels of inorganic phosphorus were decreased from 5.25 mg. before, to 3.68 mg. per 100 ml. after T.P. administration. Coinciding with the clinical improvement there was increased eosinopenic responsiveness during the intramuscular ACTH test (Table 2), and a normal response was obtained after the test was ended. That is, the ACTH resistance seemed to disappear. On the other hand, in Patient KNK there was no improvement in either symptoms or eosinopenic responsiveness to intramuscular ACTH; headache became rather severe, and he complained of sleeplessness.

DISCUSSION

The data indicate that there was eosinophil unresponsiveness to intramuscular ACTH in some of our cases of acromegaly. Recently Forsham and his associates (7, 8) reported that several patients receiving repeated courses of certain types of ACTH (Armour) had shown increasing unresponsiveness to the hormone when it was given by intramuscular injection. Occasionally, there was a complete lack of response. Although a constant intramuscular drip of the same material failed to activate the adrenal cortex, a slow intravenous infusion over an eight-hour period was found to induce a normal response. This phenomenon has been termed "ACTH resistance" by Forsham. Our cases were not treated with ACTH or any other hormonal preparation previously, in contrast with those of Forsham *et al.* It is of particular interest, however, that there are phenomena which may well be termed "ACTH resistance" in acromegaly, *i.e.*, ACTH resistance in acromegaly may be a natural occurrence concerned with the pathophysiologic conditions of this disease. Renold *et al.* (8) suggested that the most important factor in "ACTH resistance" is local inactivation by simple tissue fixation or actual destruction, and that individual adrenocortical reserve, speed of local absorption of ACTH, and the possible presence of circulating antihormones or antibodies are additional factors.

As previously suggested, ACTH unresponsiveness in the case of Patient TNG may have been due to insufficient adrenocortical reserve, but in the other patients it was apparently not the result of impaired function of the adrenal cortex. Moreover, in acromegaly, adrenocortical insufficiency has been reported as a rare complication (12). Renold's observation that there was a marked loss of ACTH potency only when the intramuscular route of injection was used indicates that circulating antibodies to ACTH or nonspecific proteins also did not play an important role in our cases of acromegaly.

The speed of local absorption of ACTH may possibly be related to factors of capillary permeability (13, 14) but, as Astwood *et al.* (15) have stated, the enzymatic destruction at the site of injection or the degree of purification of ACTH have to be taken into consideration. Wolfson (16) described three types of adrenocorticotropin, ACTH-corticotropin (crude ACTH), ACTX-corticotropin (purified ACTH) and ACTIDE-corticotropin (ACTH-peptide). They differ chiefly in the degree in which they undergo inactivation when given subcutaneously or intramuscularly. ACTIDE is most susceptible to extravascular inactivation; normal eosinopenic response is observed when ACTX (purified ACTH) is injected into an ACTH-resistant patient. Furthermore, since Wolfson proposed that ACTIDE, which has no carrier protein, is even more susceptible to extravascular inactivation than is crude ACTH, it is unlikely that any properties of a protein carrier explain the susceptibility to local extravascular inactivation, and it is also strong evidence against an important participation of proteolytic enzymes or of co-factors which activate tissue proteases. The work of Choen *et al.* (17) showed, however, that enhanced effectiveness of ACTH was observed when it was administered mixed with phosphorylated hesperidin, which has antienzymatic properties. There is some discussion about the enzymatic destruction of ACTH in blood (18), and Geschwind and Li (19) suggested that the inactivation of ACTH might be attributed to a binding or absorption of the hormone to the particular tissue. Local extravascular inactivation of ACTH in our cases might be attributable to one of the biochemical or biophysical mechanisms described.

It is of great interest that a normal eosinopenic response to intramuscular ACTH was observed in 1 of our cases of acromegaly after successful treatment with T.P. According to Kinsell *et al.* (20), active acromegaly is characterized by a high serum level of inorganic phosphorus and by a high plasma level of pituitary growth hormone. Androgenic steroids will decrease the concentration of serum inorganic phosphorus and of plasma growth hormone. In general, ACTH and growth hormone are mutually antagonistic (21, 22). In regard to cortisone and growth hormone, Selye

(23, 24) has postulated the antagonistic effect in certain experiments. Moreover, there are many reports which illustrate the hormonal imbalance in acromegaly (25–28). Although we have not assayed these hormones, the concomitant increase in eosinopenic responsiveness to intramuscular ACTH and improvement in clinical symptoms during treatment with T.P. could be dependent upon decreased production of growth hormone from the pituitary. In addition, in view of experiments indicating that androgen depresses adrenocortical function (29), an increase in this function was apparently not a factor in the improvement in Patient KID during treatment with T.P. Therefore, in the "ACTH resistance" of acromegaly, an excess of growth hormone and the associated hormonal imbalance may play an important role in the process of inactivation of ACTH in the peripheral tissues. Further investigations are now under way to clarify these points.

SUMMARY

Lack of eosinopenic response to the intramuscular administration of ACTH was observed in 6 cases of typical acromegaly. On the contrary, when ACTH was given to 4 of these patients, intravenously, an eosinopenic response occurred in 3. In the patient who showed no eosinopenic response, there was a positive result with the Robinson-Power-Kepler test and a diminished urinary excretion of 17-ketosteroids. Thus impaired adrenocortical function was suspected only in this case.

Improvement in symptoms and enhanced eosinopenic responsiveness to intramuscular ACTH were also observed in 1 patient treated with testosterone propionate, a steroid which is known to inhibit the release or production of growth hormone by the pituitary.

It is suggested that "ACTH resistance," *i.e.*, extravascular inactivation, may be caused by overproduction of growth hormone by the pituitary in certain cases of acromegaly.

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THE NATURE OF ADRENALINE AND NORADRENALINE IN NORMAL HUMAN URINE*

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HOLTZ *et al.* (1), using acid hydrolysis, demonstrated the presence of a pressor substance (urosympathin) in the urine of normal man. Closer examination of the urine extract indicated that this pressor substance was a mixture of adrenaline (A), noradrenaline (NA), and the relatively inactive hydroxytyramine (2, 3). Von Euler *et al.* (3) separated these amines by means of chromatographic techniques and showed that the normal amounts of these substances were 0.1–0.2 mg. per day for hydroxytyramine, 20–60 μg. per day for NA, and 1–8 μg. per day for A.

Studies were undertaken to examine the nature of the NA and A present in human urines by (a) acid hydrolysis, (b) β-glucuronidase hydrolysis, and (c) incubation with Mulase P which was used as a source of phenolsulfatase.

METHODS

Acid hydrolysis was carried out by taking urine volumes equivalent to a one-hour excretion, acidifying with sulfuric acid to pH 1.5, and then boiling on an electric hot plate for five, fifteen or thirty minutes. After rapid cooling, the urine was extracted.

β-Glucuronidase[1] hydrolysis was carried out by the method described by Silber and Porter (4). This consisted of adding sufficient enzyme to the urine to make a 1 per cent solution and then incubating at pH 6.5 for two hours at 37° C. All samples were equivalent to a one-hour excretion of urine.

The Mylase[1] P incubation, which was chosen as a source of phenolsulfatase, was accomplished by the method described by Cohen and Bates (5). This consisted of making various Mylase P concentrations (0.1 to 5.0 per cent) with urine and incubating at a temperature of 50° C. at pH 6.0.

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[1] Bacterial β-glucuronidase (21,500 units per gram) was obtained from Sigma Chemical Company, St. Louis, Missouri. Mylase P was a product of Nutritional Biochemicals Corp., Cleveland, Ohio.

Catechol amines were extracted by the alumina absorption method of von Euler and Hellner (2). After each hydrolysis procedure, 1 cc. of 20 per cent aluminum sulfate was added per 100 cc. of urine; 0.5 N NaOH was added with continuous stirring, to pH 7.6. The precipitate of aluminum hydroxide was allowed to settle. The precipitate was separated by centrifugation after washing twice with distilled water and then dissolved in 2 N H_2SO_4. After bringing the solution of the dissolved precipitate to pH 3.5 with 0.5 N NaOH, four volumes of equal parts of acetone and ethanol were added in order to separate out the salts. The solution was left in the refrigerator overnight. It was then filtered and evaporated *in vacuo* to a volume of 2–3 cc. The final extract was adjusted to pH 4.0. This preparation was used for the bioassay and it was found to be stable for at least four days in the ice box.

The bioassay was performed by a modification of the method described by Gaddum and Lembeck (6). This method consisted of testing the sample on the rat colon for NA, and on the rate uterus for A. This bioassay is based on the quantitative inhibition by the amines of the contraction induced *in vitro* in a 2-cc. bath with acetylcholine (7, 8). The actions of A and NA on the colon are approximately equal, whereas on the uterus A is 75–300 times more potent than NA. When the total NA or A value was desired, the colon assay was considered sufficient.

RESULTS

Table 1 presents the data of seven experiments in which four one-hour urine aliquots were taken from each urine sample. One aliquot was extracted directly; the second, third and fourth were acidified and boiled for five, fifteen, and thirty minutes respectively, and then extracted. Experiment No. 335 represents the data on total A and NA which were obtained by the rat-colon assay. All other experiments were carried out on both the rat uterus for A, and the rat colon for NA. It may be noted that acid hydrolysis increased the NA titer after five minutes of boiling; there was little loss after fifteen minutes of boiling; but thirty minutes of boiling

TABLE 1. NA AND A VALUES AT INTERVALS DURING ACID HYDROLYSIS OF URINE
(No. of minutes boiled at pH 1.5.)

Sample No.	0		5 min.		15 mins.		30 mins.	
	NA μg.	A μg.	NA μg.	A μg.	NA μg.	A μg.	NA μg.	A μg.
335*	2.3	—	4.9	—	4.4	—	2.4	—
358	3.0	.09	5.0	.11	3.9	.12	3.0	.06
364	1.5	.02	3.9	.02	3.2	.02	1.9	.01
489	2.6	.43	2.1	.40	3.0	.78	2.1	.74
499	1.0	.02	2.3	.02	1.2	.01	0.9	.01
501	1.4	.35	2.3	.39	2.1	.29	1.9	.27
502	3.3	.07	3.7	.14	2.7	.08	2.3	.11

* Total adrenaline plus noradrenaline colon assay.

TABLE 2. NA AND A VALUES AFTER β-GLUCURONIDASE HYDROLYSIS

Sample	Unhydrolyzed		β-Glucuronidase (1%) at pH 6.5 for 2 hrs.	
	NA μg.	A μg.	NA μg.	A μg.
1*	2.6	—	4.8	—
2	2.9	.33	4.7	.31
3	1.9	.37	3.5	.23
4	3.3	.40	5.5	.32
5	3.2	.03	5.6	.02
6	1.0	.18	3.0	.16
7	0.7	.05	1.7	.04

* Total adrenaline plus noradrenaline colon assay.

resulted in the loss of up to 50 per cent compared to the high values obtained after five minutes of boiling. The A appeared to be free. Experiment No. 489 shows an increase in measurable A after fifteen minutes of boiling. Though the increase in amount was small percentagewise, it was appreciable. The NA was apparently combined up to 60 per cent.

Table 2 presents data of the NA and A after 1 per cent β-glucuronidase hydrolysis. There was a marked rise in the measurable amount of NA. The A remained the same, indicating that it was possibly free.

Table 3 presents data comparing the amounts of NA and A released after glucuronidase and after acid hydrolysis (fifteen minutes). The enzyme hydrolysis yielded higher values than did the acid hydrolysis.

Experiments conducted with β-glucuronidase in a concentration of 0.1 per cent showed no increase in the measurable amount of NA.

To test the hypothesis that the conjugated material may be a phenol-sulfate, Mylase P was used in a concentration of 2 per cent. Table 4

TABLE 3. COMPARISON OF ACID HYDROLYSIS (15 MIN.) AND β-GLUCURONIDASE (1%) HYDROLYSIS AT PH 6.5 FOR TWO HOURS

Sample No.	Unhydrolyzed		Acid hydrolysis		β-Glucuronidase	
	NA μg.	A μg.	NA μg.	A μg.	NA μg.	A μg.
375	2.9	.33	3.5	.38	4.7	.31
436	1.9	.37	2.2	.46	3.5	.23

TABLE 4. NA AND A VALUES AFTER MYLASE P HYDROLYSIS
(Incubation for two hours)

Sample No.	Procedure	NA μg.	A μg.
459	A. 2% Mylase P	1.4	.06
	B. Unincubated control	1.6	.08
460	A. 2% Mylase P	2.7	.06
	B. Incubated control	2.3	.02
481	B. 2% Mylase P	4.0	.05
	C. 1% β-Glucuronidase	5.6	.02
	D. Incubated control	3.2	.03
496	A. Incubated control	2.2	.31
	B. 0.5% Mylase P	2.6	.29
	C. 2.0% Mylase P	2.8	.30
	D. 5.0% Mylase P	2.0	.21
523	A. Incubated control	2.7	.19
	B. 2.0% Mylase P	2.3	.15
	C. 0.5% Mylase P	2.6	.14
	D. 0.1% Mylase P	2.1	.12

shows the results when the incubation was carried out for two hours. No increases could be observed in either NA or A. In Experiment No. 481, when aliquots of the same urine samples were treated by glucuronidase and Mylase P, there was confirmation of the concept that the conjugate of NA is a glucuronide. When various concentrations of Mylase P were used up to 5 per cent (Exp. Nos. 496 and 523), the results were negative. When the incubation was carried out for four and for twenty hours, the NA and A activities were lost (Table 4).

TABLE 5. NA AND A VALUES AFTER MYLASE P INCUBATION AT PH 6.0

Time incubated	Mylase P (2.0%)		Incubated control	
	NA μg.	A μg.	NA μg.	A μg.
2 hrs.	3.3	.17	—	—
4 hrs.	1.4	.12	2.9	.15
20 hrs.	0.7	.03	2.3	.04

DISCUSSION

The early studies on the nature of A in the urine were conducted only after the administration of A, due to the fact that the techniques then used revealed no A in normal urine. The first extensive study on the subject by Richter (9) indicated that, after oral administration in the human, up to 70 per cent of A appeared as a conjugate in the urine and that the conjugate was the sulfate. This was confirmed in terms of biologic activity by Richter and MacIntosh (10); and Beyer and Shapiro (11) demonstrated the presence of a conjugate in the urine of the dog after A administration. The conjugate was also demonstrated in the urine of the rabbit, but the evidence indicated that the conjugate was a glucuronide and not the sulfate (12, 13). The data obtained in these studies involved the administration of large unphysiologic doses, usually given orally, and do not shed any light on the nature of the A in normal urine.

More recently Schayer (14) demonstrated that when rats were injected with small doses of C^{14} A, the A appeared in the urine in the free form. However, when large doses were administered, the amine appeared in both the free and conjugated form. Furthermore, when the A was administered orally, practically all of the compound appeared in the combined form (15).

Von Euler and Hellner (2) extracted the urine sample directly, and then hydrolyzed the filtrate and re-extracted it. An additional 56 per cent of NA and 12 per cent of A were obtained after the hydrolysis. Since the extraction procedure is only 70–80 per cent efficient, it is possible that the small increase of 12 per cent in A after hydrolysis is not significant enough to indicate further release of A. The unextracted portion (20–30 per cent) could easily account for the increase on the second extraction. Furthermore, such a small increase as 12 per cent might be accounted for by the margin of error inherent in the hydrolysis and bioassay methods used. The increase of the NA after hydrolysis is marked and the data in this paper confirm the proportion of NA which appears to be combined.

SUMMARY

Studies were conducted to determine the nature of adrenaline (A) and noradrenaline (NA) present in normal human urine. In addition to acid hydrolysis, the urine was incubated with β-glucuronidase and with Mylase P as a source of phenolsulfatase.

1. With acid hydrolysis, the highest values of NA were obtained after five minutes of boiling at pH 1.5.

2. Definite loss of NA and A activity was observed after boiling for periods of fifteen to thirty minutes.

3. NA is conjugated to the extent of 60 per cent, whereas A is in all likelihood free.

4. The conjugate of NA in normal urine appears to be the glucuronide and not the sulfate.

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EXCRETION OF ADRENALINE AND NORADRENALINE IN HUMAN SUBJECTS*

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THE pressor substance in normal human urine demonstrated by Holtz *et al.* (1) was considered by them to be a mixture of adrenaline, noradrenaline and hydroxytyramine. A few years later Von Euler *et al.* (2, 3) succeeded in separating all three amines from urine extracts by the use of chromatographic techniques.

In a preceding paper (4) we reported observations regarding the nature of the adrenaline and noradrenaline in normal human urine. The present report concerns experiments which were conducted to determine the excretion of adrenaline (A) and noradrenaline (NA): 1) in sleep and in waking states; 2) after the infusion of A and NA into an adrenalectomized subject; 3) after psychomotor stress, operating the Hoagland-Werthessen pursuitmeter (5) under normal and hypoxic conditions; and 4) after the administration of ACTH.

METHODS

The extraction of A and NA was accomplished by the alumina adsorption method of von Euler and Hellner (2). A 1-hour to 2-hour equivalent of urine collection was brought to pH 1.5 with sulfuric acid and boiled on an electric hot plate for twenty minutes. After cooling rapidly, 1 cc. of 20 per cent aluminum sulfate per 100 cc. of urine was added; then 0.5 N NaOH was added, with continuous stirring, to pH 7.6 The precipitate of aluminum hydroxide was allowed to settle. The precipitate was separated by centrifugation after washing twice with distilled water, and dissolved in 2 N H_2SO_4. After adjusting the solution to pH 3.5 with 0.5 N NaOH, four volumes of equal parts of acetone and alcohol were added and the material was placed in the icebox overnight in order to separate out the salts. It was then filtered and evaporated *in vacuo* at 45° C. to a volume of 2 or 3 cc. The final extract, which was free of organic solvents, was then adjusted to pH 4.0. This preparation was used for the bioassay, and it was found to be stable for at least four days when kept in the icebox.

The bioassay was performed by a modification of the method described by Gaddum

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and Lembeck (6). This method consisted of testing the sample on the rat colon for NA, and on the rat uterus for A. The bioassay is based on the quantitative inhibition by the catechol amines of the contractions induced *in vitro* in a 2-cc. bath with acetylcholine (7, 8). The inhibitions of NA and A are approximately equal when tested on the colon; but when tested on the uterus, A is 75 to 300 times more potent than NA. The colon assay was used when quick estimates of total (NA+A) were desired.

RESULTS AND DISCUSSION

Sleep and waking conditions

Both normal and psychotic individuals were studied. Night samples of urine were collected during a period from approximately 10:00 P.M. to 6:30 A.M., and day samples were collected from 6:30 A.M. to noon. All subjects had breakfast and conducted their usual activities which, for the normal subjects, consisted of laboratory routines.

Preliminary experiments were carried out on samples assayed by the rat-colon method, which furnishes an estimate of the total NA plus A. Five samples of day urine were extracted in duplicate and the differences be-

TABLE 1. TOTAL URINARY ADRENALINE (A) PLUS NORADRENALINE (NA): COMPARISON OF DUPLICATE DAY SAMPLES VERSUS COMPARISON OF NIGHT AND DAY SAMPLES

Sample No.	Nature of collection	Total NA+A* (μg./hr.)
U 1 A	(D)†	6.4
B		4.4
U 6 A	(D)	1.9
B		1.7
U 7 A	(D)	2.0
B		2.2
U 8 A	(D)	3.0
B		3.4
U 9 A	(D)	1.0
B		0.9
U 3 F.E.	(N)	0.6
U 4 F.E.	(D)	4.4
U 10 E.L.	(N)	1.2
U 11 E.L.	(D)	2.8

* Colon assay: estimate of total adrenaline plus noradrenaline.

† (D) = Day sample (*see* text). (N) = Night sample.

tween duplicates were compared with the differences observed between the sleep (night) and waking (day) samples of 2 normal subjects (Table 1). Fair to good agreement was obtained in the duplicate samples, but there was much more NA+A in the day samples than in the night sample of the 2 individuals tested.

In Table 2 are presented data on the distribution of the amines during

TABLE 2. DISTRIBUTION OF ADRENALINE (A) AND NORADRENALINE (NA) IN NIGHT AND DAY SAMPLES OF URINE

Subject	Type of collection	NA (μg./hr.)	A (μg./hr.)	A (%)
F.F.	(N)*	0.8	0.05	5.0
	(D)	1.9	0.41	17.5
S.W.	(N)	1.0	0.01	1.0
	(D)	1.6	0.04	2.4
F.E.	(N)	1.4	0.01	1.0
	(D)	1.2	0.03	2.4
S.K.	(N)	0.9	0.03	3.0
	(D)	1.8	0.15	7.9
N.G.	(N)	1.4	0.02	1.3
	(D)	4.4	0.48	9.8
E.F.	(N)	1.8	0.05	2.7
	(D)	4.6	0.12	2.6
J.D.†	(N)	1.1	0.08	7.2
	(D)	3.1	1.05	25.6

* (N) = Night sample. (D) = Day sample (*see* text).
† Acute schizophrenia.

sleep and waking states in 6 normal subjects and 1 male schizophrenic patient. There was an increase in the excretion rate of A in each case, but there was a wide range of values. The NA values were more uniform and increases were not as large percentagewise as for A values.

Table 3 shows a comparison of A, NA, and 17-ketosteroid (17-KS) excretion in night and day samples of 4 normal subjects, 2 male schizophrenics, and 1 adrenalectomized female psychotic patient. The adrenalectomized patient was maintained on 40 mg. of cortisol a day, orally, with 2 Gm. of sodium chloride. The data are expressed in terms of 100 mg. of creatinine.

In the subjects with intact adrenals there was a diurnal variation in all three indices, A, NA, and 17-KS. Increases over the values for the night samples were greatest for A, with NA and 17-KS values following in that order. The adrenalectomized subject, C.P., did not excrete A, but there was a marked increase in NA excretion during the day compared with that

TABLE 3. URINARY EXCRETION OF ADRENALINE (A), NORADRENALINE (NA) AND 17-KETOSTEROID: NIGHT VERSUS DAY

Date	Subject	Type of collection	NA, μg. per 100 mg. creatinine	A, μg. per 100 mg. creatinine	17-KS, mg. per 100 mg. creatinine
Dec. 20, '53	F.F.	(N)*	2.3	0.05	0.49
		(D)	4.5	0.82	0.58
Dec. 19, '53	E.L.	(N)	1.6	0.06	0.69
		(D)	3.7	0.74	1.00
Jan. 5, '54	E.L.	(N)	0.9	0.03	0.69
		(D)	7.2	1.98	1.17
Dec. 19, '53	A.Z.	(N)	1.9	0.10	0.63
		(D)	4.6	0.78	0.87
Dec. 7, '53	R.S.†	(N)	3.0	0.30	0.90
		(D)	8.0	1.08	1.33
Dec. 9, '53	R.L.†	(N)	4.1	0.06	0.65
		(D)	6.4	0.44	2.41
Jan. 15, '54	C.P.‡	(N)	0.8	x§	xx‖
		(D)	4.4	x§	xx‖

* (N) = Night sample. (D) = Day sample (*see* text).
† Schizophrenic patients.
‡ Adrenalectomized psychotic female.
§ None detected.
‖ Analysis not performed.

during the night. No A was detected in a 24-hour sample from this patient, nor from another adrenalectomized female psychotic patient (P.M.). If A were present, its rate of excretion would have been less than .01 μg. per hour.

Diurnal variation of adrenocortical function was first demonstrated by Pincus, measuring the 17-KS excretion (9). Though changes in the sym-

pathico-adrenal system with respect to sleep and waking have been known for some time, biochemical data have been lacking. The demonstration of the increase in A and NA excretion in waking over that of sleep does not mean that these changes are due to a diurnal rhythm independent of the general physical and psychologic factors involved in waking. The process of waking may be considered as a stress, compared to sleeping as a control. These data indicate that the percentile increase of A is greater than the percentile increase of NA, though the value for the excretion rate of the latter is greater in terms of μg. per hour. In a few experiments both sympathico-adrenal (A and NA) and adrenal-pituitary (17-KS) functions were measured on the same urines. The results indicated that, though both measurements showed an increase upon waking compared with those during sleep, there was no discernible quantitative relationship.

Infusion studies in an adrenalectomized patient

Five infusion experiments were conducted on an adrenalectomized psychotic female patient, (P.M.), weighing 110 Kg. The results are presented in Table 4. There were three infusions of A and two of NA. For the purpose of discussion, the amount of A or NA appearing in the infusion

TABLE 4. INFUSION OF ADRENALINE (A) AND NORADRENALINE (NA) INTO AN ADRENALECTOMIZED PATIENT (P.M.)

Exp. No.	Date	Total dose (μg.)	Infusion time and duration	Collection of urine	A μg./hr.	NA μg./hr.	NA+A in infusion collection (%)
1	Feb. 16, '54	202 A	3:32 P.M. for 15 min.	(C)* 12:50–3:30 P.M.	None	5.2	
				(I) 3:30–4:25 P.M.	4.02†	Lost	1.7
2	Mar. 1, '54	165 A	2:55 P.M. for 15 min.	(C) 1:45–2:45 P.M.	None	2.2	0.5
				(I) 2:45–3:45 P.M.	0.84	3.6	
				(P) 3:45–5:00 P.M.	0.01	2.2	
3	Mar. 8, '54	165 A	2:15 P.M. for 15 min.	(C) 11:35 A.M.–1:55 P.M.	None	1.5	0.5
				(I) 1:55–3:15 P.M.	0.78	2.1	
				(P) 3:15–4:30 P.M.	0.03	2.9	
4	Mar. 23, '54	165 NA	2:20 P.M. for 15 min.	(C) 12:00–2:00 P.M.	None	3.9	
				(I) 2:00–2:45 P.M.	None	15.3	5.7
				(P) 2:45–4:30 P.M.	None	6.0	
5	Jan. 19, '55	80 NA	11:00 A.M. for 10 min.	(C)‡ 10:00–10:55 A.M.	None	2.5	
				(I) 10:55–12:00	0.06	5.5§	3.4
				(P) 12:00–1:00 P.M.	None	1.6	

* (C) = Control.
(I) = Infusion.
(P) = Post-infusion collection.

† Unhydrolyzed urine titer was 3.52 μg./hr. for A.

‡ All samples were hydrolyzed by boiling 15 min. at pH 1.5 except the (I) sample of experiment 5, which was hydrolyzed with 1% beta-glucuronidase (215 units/cc. of urine) for 2 hours at 37°C. and pH 6.5.

§ Unhydrolyzed urine titer was 4.43 μg./hr. for NA in unhydrolyzed sample.

(I) urine sample (the control (C) values being zero) was considered as the amount of the total dose appearing in the urine.

The total dose of A in Experiment 1 was larger than intended, as the rate of infusion was almost 0.2 μg./Kg./min. for the first five minutes. The dose was reduced and the total A administered in the fifteen-minute period was 202 μg. The infusion collection contained only 1.7 per cent of the total infusion dose. With a smaller and more uniform infusion rate of 0.1 μg./Kg./min. in Experiments 2 and 3, the results were somewhat similar. The total dose of A in each case was 165 μg. and only 0.5 per cent of the total A administered was measurable in the infusion sample. During the NA infusions shown in Experiments 4 and 5 a larger percentage of the dose appeared in the urine. When the dose was 165 μg. (0.1 μg./Kg./min.) the infusion collection contained of 5.7 per cent of the total dose, and with the smaller dose of 80 μg. (0.05 μg./Kg./min.) the urine contained 3.4 per cent of the total dose. Two experiments conducted to determine the nature of the amine appearing in the urine after infusion indicate that it is mostly free (Table 4).

The experiments of Table 4 indicate that in the adrenalectomized patient a far greater percentage of NA appears in the urine after NA infusion (3.4 per cent with an 80-μg. dose, and 5.7 per cent with an 165-μg. dose) than of A after A infusion (1.7 per cent with a 202-μg. dose and 0.5 per cent with a 165-μg. dose). These results are consistent with the infusion data published by von Euler and Luft (10) on subjects with intact adrenals. If, for the purpose of approximating the secretion rate of A by the adrenal medulla, the value of 0.5 per cent be taken as the proportion appearing in the urine of the total secreted by the gland in a one-hour period, it may be estimated that the normal rate of secretion is 20 μg. per hour, when 0.1 μg. per hour is taken as the normal rate of excretion. Since the percentage excretion is related to the dose infused (Table 4), the value of 0.5 per cent may still be too high. Similarly, if the figure of 3.4 per cent be accepted as the proportion appearing in the urine of the total secretion of NA by the sympathico-adrenal system, it may be estimated that the rate of secretion is 60 μg. per hour, when 2.0 μg. per hour is taken as the normal rate of excretion.

The data on the control urines of the adrenalectomized Patient P.M. show the lack of A in the urine before infusion. Thus the NA in the control urines must come from extra-adrenal sources.

Psychomotor stress

Three-hour studies. Table 5 shows data on the 4 normal subjects (aged 26 to 34) who operated the Hoagland-Werthessen pursuitmeter for three hours. They had rest periods of five minutes at the end of the first and

TABLE 5. ADRENALINE (A) AND NORADRENALINE (NA) EXCRETION IN PSYCHOMOTOR STRESS WITHOUT HYPOXIA (HOAGLAND-WERTHESSEN PURSUITMETER)

Subject	Date	A μg./hr.	NA μg./hr.
N.G.	Jan. 26, '54	(C)* 0.17	4.2
		(S) 1.13	7.6
	Feb. 2, '54	(C) 0.35	4.2
		(S) 0.93	6.6
	Feb. 9, '54	(C) 0.48	4.4
		(S) 2.00	3.8
S.K.	Jan. 27, '54	(C) 0.06	2.7
		(S) 0.07	5.9
	Feb. 3, '54	(C) 0.08	1.7
		(S) 0.10	2.7
	Feb. 8, '54	(C) 0.15	1.8
		(S) 0.14	3.4
F.U.	Feb. 15, '54	(C) 0.18	4.0
		(S) 0.23	—
	Feb. 23, '54	(C) 0.24	1.5
		(S) 0.24	1.7
	Mar. 2, '54	(C) 0.48	2.0
		(S) 0.68	2.5
T.H.	Feb. 26, '54	(C) 0.15	2.0
		(S) 0.37	2.6
	Mar. 5, '54	(C) 0.14	0.9
		(S) 0.16	1.5
	Mar. 12, '54	(C) 0.09	2.8
		(S) —	—

* (C) = Control. (S) = Stress (*see* text).

second hours. The pre-stress control sample represents urine collected during a period approximately between 8:00 A.M. and 1:00 P.M.; the stress sample represents urine collected during the operation of the pursuitmeter, *i.e.*, from 1:00 P.M. to 4:00 P.M.

Subject N.G. showed a marked response to stress in A excretion in each of the three experiments. On the other hand Subject S.K. showed little, if any, increase in A excretion but fair increases in NA. Subjects T.H. and F.U. showed, in general, small increases in both A and NA excretion. Though precautions were taken to control the factor of motivation by awarding prize money for the best performers, it is possible that the results were influenced by the relative effort made by each subject in the psychomotor performance. It is of interest that Subject N.G. was determined to be the best performer and actually succeeded in being the highest scorer, whereas Subject S.K. was indifferent and was the lowest scorer. The number of experiments was too small to serve as a basis for generalizations; suffice it to state that consistent increases in A or NA, or both, were demonstrated in the 4 subjects tested with this procedure for inducing psychomotor stress.

Two-hour studies. Six young men (aged 17 to 19) were each subjected to from three or four psychomotor-stress tests under hypoxic conditions (10 per cent oxygen) for two hours. Control urines were collected in the forenoon, as in the three-hour group. The experiments were conducted from 1:00 P.M. to 3:00 P.M., and the subjects did not have any rest during the stress period.

Under these conditions, considered as a group, there was a significant increase in the output of A during the stress period, compared with the control output in 17 runs in which both were determined (Table 6). The mean value of the control NA excretions in 18 experiments was 2.26 μg. per hour as compared with 2.73 μg. per hour for the stress samples. The mean value of the control A excretions in 17 experiments was 0.079 μg. per hour as compared with 0.174 μg. per hour for the stress samples. The increase in the excretion of A during stress was significant: $P = <.01$. However, it was evident that there were individual differences. Subject P.P. showed a marked increase in A in each of his three runs, whereas Subject R.P., his identical twin, showed no increase during the same procedure. Subjects M.W. and P.D. showed fair to distinct increases in each of their three runs, whereas Subjects J.B. and R.H. showed little if any increase during their early tests, but a definite increase during the later experiments.

As a group, there was a significant increase in the output of A but not of NA. The individual differences could not be explained on the basis of the scores attained by the subjects in the stress procedure.

There was great variability in the excretion of A during psychomotor stress. The variability was much less for NA. These features were also observed by von Euler and Hellner (11) in the study of physical stresses, such as running, and by Franksson *et al.* in a study of surgical trauma (12). During psychomotor stress the percentagewise increase in excretion

TABLE 6. ADRENALINE (A) AND NORADRENALINE (NA) EXCRETION IN PURSUIT-METER STRESS WITH HYPOXIA

Subject	Age (yrs.)	Date	A μg./hr.	NA μg./hr.
P.P.	17	Jly. 19, '54	(C)* 0.10	1.4
			(S) 0.42	1.6
		Aug. 3, '54	(C) 0.03	3.2
			(S) 0.26	5.2
		Aug. 31, '54	(C) 0.04	2.0
			(S) 0.35	3.4
R.P.	17	Jly. 15, '54	(C) 0.14	1.5
			(S) 0.16	1.5
		Aug. 6, '54	(C) 0.09	3.0
			(S) 0.05	1.6
		Aug. 27, '54	(C) 0.16	2.2
			(S) 0.17	1.5
M.W.	17	Jly. 16, '54	(C) —	1.2
			(S) —	2.4
		Aug. 2, '54	(C) 0.07	2.8
			(S) 0.25	6.0
		Aug. 9, '54	(C) 0.09	5.0
			(S) 0.19	3.3
P.D.	16	Oct. 18, '54	(C) 0.07	2.4
			(S) 0.15	4.9
		Nov. 29, '54	(C) 0.01	1.2
			(S) 0.11	1.5
		Dec. 13, '54	(C) 0.12	0.8
			(S) 0.18	4.5
J.B.	17	Oct. 19, '54	(C) 0.12	1.9
			(S) 0.09	1.0
		Nov. 30, '54	(C) 0.03	1.7
			(S) 0.03	1.0
		Dec. 14, '54	(C) 0.06	3.8
			(S) 0.14	3.8
		Dec. 20, '54	(C) 0.04	1.7
			(S) 0.10	2.5
R.H.	18	Oct. 29, '54	(C) 0.08	2.5
			(S) 0.05	1.6
		Dec. 8, '54	(C) 0.08	2.5
			(S) 0.26	2.1
		Dec. 29, '54	(C) —	—
			(S) 0.20	4.0

* (C) = Control. (S) = Stress.

was much greater for A than for NA. There may have been individual variation in the degree of psychomotor stress, due to the factor of motivation and the effort made to perform on the pursuitmeter. When, in addition to stress, there was hypoxia, a significant increase occurred in A excretion compared with that of the control period.

Though the increase in the output of A in some cases was tenfold, this should not be interpreted as an indication of a similar increase in the magnitude of secretion. The infusion data show that the percentage of the infused amine appearing in the urine is dependent on the dose given per unit of time. Thus, with a twofold increase in the dose one might expect a fourfold to sixfold increase in the rate of urinary excretion.

After injection of ACTH

Experiments were conducted on 4 normal subjects who received 20 units of ACTH for three consecutive days, preceded and followed by control periods. Determinations of NA, A, sodium, potassium and 17-KS were carried out on 24-hour urine samples.

The results are listed in Table 7. NA and A excretions are expressed in terms of μg. per twenty-four hours, 17-KS as mg. per twenty-four hours, and sodium and potassium as the weight ratio K/Na. In each experiment there was a pronounced decrease in the NA titer after ACTH except for day 4 of Subject N.G. In all instances there was a diminution of NA excretion during administration of ACTH and the day following the last injection. The excretion of A was variable and was not related to the ACTH administered. 17-KS excretion was markedly increased during the ACTH injection period and the K/Na ratio was elevated. The K/Na ratio showed a marked depression in each case when the ACTH was stopped, due, for the most part, to the increased sodium excretion. Whether these findings were the results of the metabolism of NA, or of alterations in the biosynthetic processes cannot be deduced from these experiments. The data confirm those presented by von Euler and Luft when ACTH was given to arthritic subjects (13, 14).

SUMMARY

A study has been conducted on the excretion of adrenaline (A) and noradrenaline (NA): 1) in sleep and in waking states; 2) after the infusion of A or NA into an adrenalectomized subject; 3) after psychomotor stress; and 4) after the administration of ACTH.

There was an increase in the excretion of A and NA in the waking state compared with that during sleep, but the percentage increase was greater for A. When sympathico-adrenal function (A and NA excretion) and pituitary-adrenal function (17-ketosteroid excretion) were measured in

TABLE 7. EXCRETION OF ADRENALINE (A) AND NORADRENALINE (NA) AFTER ADMINISTRATION OF ACTH TO 4 NORMAL SUBJECTS

Subject	Day	NA (μg./24 hrs.)	A (μg./24 hrs.)	17-KS (mg./24 hrs.)	K/Na (wt. ratio)
C.V.	1	54.96	1.75	9.65	.48
	2	89.28	—	10.00	.53
	3*	36.48	.95	15.90	.87
	4*	23.76	1.73	20.00	.68
	5*	23.52	.48	14.70	.59
	6	21.84	—	20.60	.22
	7	58.08	.75	12.40	.38
	8	73.44	.93	14.20	.38
F.M.	1	82.3	.89	12.5	.74
	2	65.3	1.87	7.4	.82
	3*	20.6	2.90	16.3	2.19
	4*	39.8	.58	25.7	1.52
	5*	25.2	1.01	20.6	1.08
	6	19.7	—	9.1	1.22
	7	33.3	.48	11.3	.27
	8	91.2	.91	12.5	.16
N.G.	1	71.8	6.05	16.6	.50
	2	85.4	9.79	15.6	1.00
	3*	40.3	1.10	26.9	2.00
	4*	83.5	2.14	23.3	.35
	5*	48.2	2.66	22.8	.62
	6	41.5	3.50	10.3	.16
	7	54.5	1.75	18.5	.23
	8	43.4	3.67	18.7	.49
E.F.	1	69.8	1.08	11.3	.52
	2	81.9	.60	11.8	.60
	3*	38.9	3.55	15.4	.88
	4*	30.2	1.03	23.5	.61
	5*	65.5	2.45	20.2	.63
	6	87.8	1.51	17.3	.27
	7	136.4	—	13.9	.49
	8	94.6	1.54	13.2	.61

* Organon Zinc Hydroxide ACTH, 20 I.U.

the same samples, both showed increases for the waking state compared to the values during sleep, but no quantitative relationships were apparent. In the 1 adrenalectomized patient studied, there was no detectable A in the urine, but there was an increase in the NA excretion in the waking state over that during sleep.

Approximately two hours after beginning an infusion of A or of NA into the adrenalectomized patient results indicated that at the dosage level of 165 μg. (0.1 μg./Kg./min.) only 0.5 per cent of the administered A, but 5.7 per cent of the administered NA appeared in the urine. The percentage of the infused amine measurable in the urine after the infusion was dependent upon the dose given per unit of time; the larger the dose the greater the percentage measurable in the urine.

Though there was great individual variability in the values obtained during the psychomotor stress of performing on the Hoagland-Werthessen-pursuitmeter, increases in the excretion of both A and NA were demonstrated. When the stress included hypoxia (17 experiments on 6 subjects), there was a significant increase in the excretion of A but not of NA compared with the values for the control sample.

The injection of 20 I.U. of ACTH daily for three days days into 4 normal subjects resulted in a decrease in the excretion of NA but little or no change in excretion of A.

Acknowledgments

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GONADAL DYSGENESIS WITH ANDROGENIC MANIFESTATIONS IN THE TALL EUNUCHOID FEMALE*†

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GORDAN *et al.* (1) recently reported a syndrome characterized by primary amenorrhea, lack of development of primary and secondary sexual characteristics, clitoridal hypertrophy, hirsutism, delayed skeletal maturation, and short stature with webbing of the neck. Except for the hirsutism and the hypertrophy of the clitoris, the clinical picture resembled that of Turner's syndrome (2). Lack of sexual development with decreased stature and high urinary FSH excretion due to primary ovarian agenesis has been described by Albright *et al.* (3), Varney *et al.* (4), and Wilkins and Fleischmann (5). However, Albright *et al.* (3) also reported 2 patients with primary ovarian insufficiency who were not short in stature and whose urinary excretion of FSH was within normal limits. The purpose of this paper is to report a case which shows features of both Gordan's and Albright's cases, though differing from each. This variant may help to fill in a link in the clarification of ovarian agenesis. The term "eunuchoid" is used because this patient resembled the tall eunuchoid male in appearance (Fig. 1). The term "dysgenesis" is employed because of the coexistence of male and female components in the rudimentary gonadal structures.

CASE REPORT

The patient was a white female, 25 years old, referred to us because of amenorrhea, lack of breast development, enlarged clitoris and extreme bodily thinness. She had first noted enlargement of the clitoris when she was 14 years old. Her height was 70 inches (175 cm.) and her span 69.6 inches (174 cm.) The blood pressure was normal. The voice was feminine. Pubic hair and axillary hair were sparse; there was neither facial hair nor acne, and there was no webbing of the neck. The labia majora were well developed. The clitoris was markedly enlarged (Fig. 2). The vaginal canal was well formed, and an infantile uterus was palpable on bimanual examination. Skeletal maturation was approximately that of a 14-year-old girl. The urinary 17-ketosteroid excretion (method of Drekter *et al.* (6)) was 4.4 and 2.6 mg. per twenty-four hours. The urinary 17-hydroxy-

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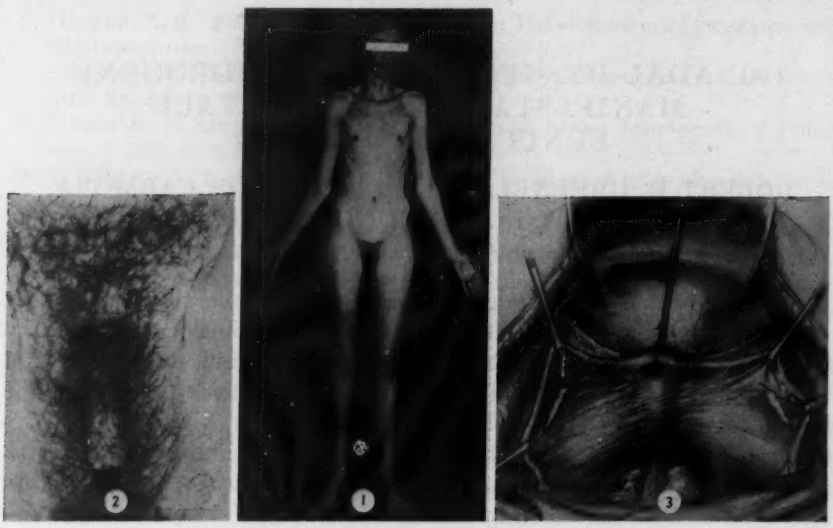


FIG. 1. Tall eunuchoid female with lack of breast development.

FIG. 2. Marked enlargement of clitoris.

FIG. 3. Operative findings: infantile uterus, elongated Fallopian tubes and rudimentary gonadal structures.

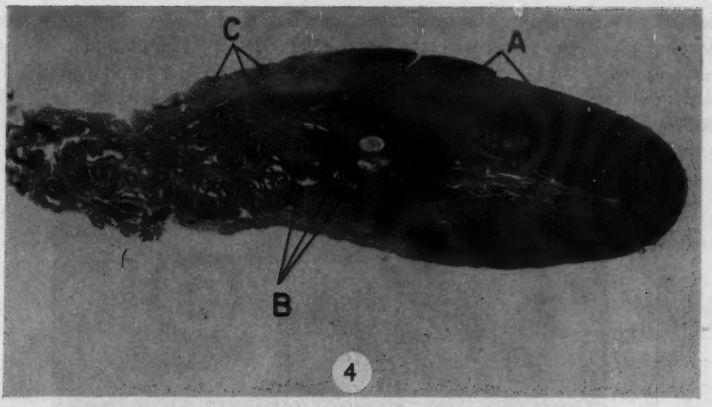
corticoid excretion (method of Reddy *et al.* (7)) was 0.5 and 3.0 mg. per twenty-four hours. The urinary excretion of FSH (method of Klinefelter *et al.* (8)) was within normal limits on one determination; on a second determination the titer was more than 52 and less than 104 M.U. (Table 1). The vaginal smear showed atrophy. The chromosomal nuclear configuration of the skin biopsy specimen was of the female type, substantiated by Dr. M. Grumbach and also by Dr. M. Barr. In the study of oral mucosal smears, a female sex chromatin pattern was noted in 79 per cent of the nuclei. X-ray examination showed the sella to be normal and the bony pelvis to be feminine in type. Exploratory laparotomy was performed and rudimentary gonadal structures, an infantile uterus, and fallopian tubes were found (Fig. 3).

Histologic findings

Histopathologic study of a cross-section of the ovary revealed typical ovarian stroma without ova or follicles. In the medullary area there was a small zone of clear polyhedral cells resembling Leydig cells surrounded by ovarian stroma (Figs. 4, 5, 6). Scattered groups of glandular structures were present in the hilus, and at the periphery was one group of glands arranged in the form of tubules. In the hilus were focal groups of slightly

TABLE 1. URINARY HORMONE ASSAYS

	Aug. 4, 1954	Jan. 28, 1955
17-Ketosteroids	4.4 mg./24 hrs.	2.6 mg./24 hrs.
17-Hydroxycorticoids	0.5 mg./24 hrs.	3.0 mg./24 hrs.
FSH	less than 52 M.U.	more than 52 and less than 104 M.U.



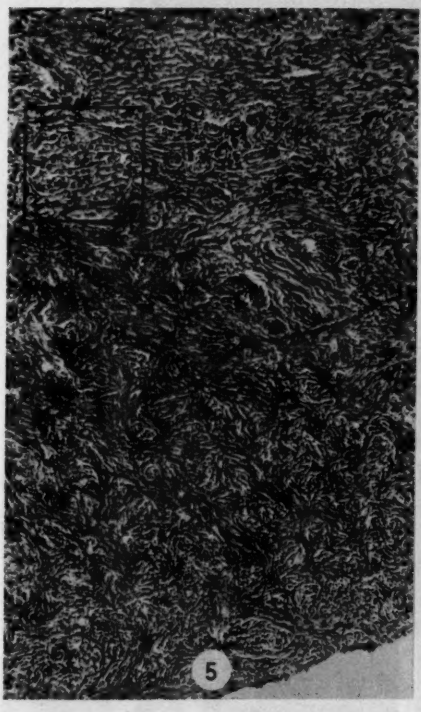
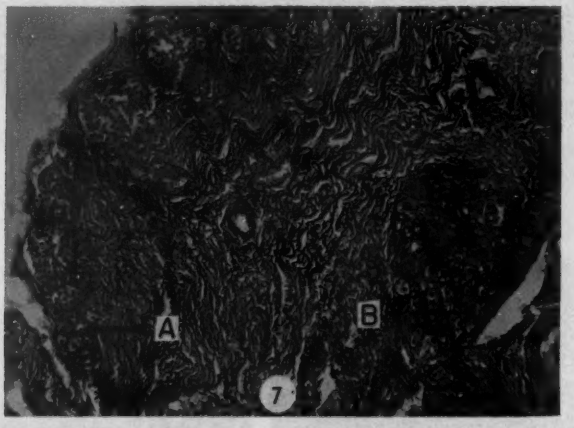


FIG. 4. Photomicrograph of cross-section of rudimentary gonad (A), showing dense stroma of ovarian cortex and glands of the rete ovarii in the hilus (B), and collection of tubular structures near periphery at C.

FIG. 5. Photomicrograph of section through cortex (A), showing ovarian stroma and absence of primordial follicles.

FIG. 6. Photomicrograph of section squared-off in Figure 5, showing collection of Leydig cells surrounded by ovarian stroma.

pigmented cells resembling the sympathicotropic cells of Berger, which are thought to be morphologically identical with the Leydig cells (9–12). The glandular structures in the hilus appeared to be remnants of the rete ovarii, but the tubular structures were probably mesonephric rests (13–16) rather than seminiferous in origin (Figs. 7, 8, 9). Reinke crystalloids were not demonstrable in the groups of Leydig cells examined.



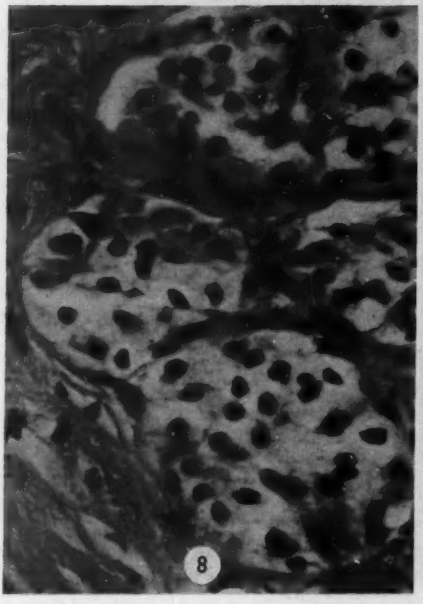


FIG. 7. Photomicrograph through peripheral section of hilus (Fig. 4C), showing tubular structures at periphery (A) and focal masses of Leydig cells in hilus (B).

FIG. 8. Photomicrograph of 7A enlarged to show tubular structures thought to be mesonephric rests.

FIG. 9. Photomicrograph of 7B enlarged to show group of Leydig cells.

DISCUSSION

Perusal of the literature reveals a few reports of ovarian agenesis plus androgenic manifestations (1, 17, 18, 19). In some of the cases hilar cells resembling interstitial cells were found (19). However, in other cases the presence of hilar cells was not associated with signs of masculinization (20, 21). In our case the low value for urinary 17-ketosteroid excretion practically eliminates these cells as a possible source of extra androgen production (12); therefore the observed hilar cells could not account for the manifestations of virilization.

Polani *et al.* (22) reported a male type of chromosomal nuclear configuration in patients with ovarian agenesis, and this was corroborated by Wilkins *et al.* (23). The clinical implication of these finding gives rise to an hypothesis that the syndrome of ovarian dysgenesis may be a form of hermaphrodism. In fact, Robert Meyer, some time ago, described a case that fits into the general category of the subject under discussion as "hermaphroditismus externus asexualis" (17).

Treatment. Since the condition may be regarded as a variety of ovarian agenesis, the treatment should be similar, *i.e.*, substitutional therapy with estrogens, or estrogens and progesterone in cyclic fashion. Whether the enlarged clitoris should be excised is a moot point. Our patient is responding, though slowly, to cyclic estrogen and progesterone therapy, in that her psyche and well being have improved and her breasts have enlarged. The clitoris appears to have decreased in size. Several menstrual periods have been induced.

SUMMARY

1. A syndrome is reported, which is characterized by rudimentary ovaries, infantile uterus, complete lack of breast development, moderate amounts of sexual and axillary hair, tall stature, clitoridal hypertrophy, a female sex chromatin pattern in the skin, normal to slightly elevated urinary FSH, low urinary 17-ketosteroids, and clinical manifestations of hypoestrinism.

2. This case may furnish a link in the relationship between the ovarian agenesis of Albright *et al.* and the ovarian dysgenesis with masculine manifestations described by Gordan *et al.*

3. The syndrome should be considered a variant of the syndrome of ovarian agenesis and perhaps a subform of hermaphrodism, and should be differentiated from the various types of heterosexual development.

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THE USE OF METHYLTESTOSTERONE TO STIMULATE GROWTH: RELATIVE INFLUENCE ON SKELETAL MATURATION AND LINEAR GROWTH*

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TESTOSTERONE is frequently used to promote growth in children of short stature (1). Although it often causes a marked increase in the rate of growth in height, it also tends to increase the rate of skeletal maturation (2). This is of importance, since adult stature is determined not only by the rate of growth but also by the duration of growth, which is a function of skeletal maturation. Growth of the long bones stops when epiphyseal fusion is completed at the skeletal age of 16 years in girls and 18 years in boys. Treatment with testosterone may cause a decrease in ultimate stature if it causes skeletal maturation to advance more rapidly than linear growth. Testosterone therapy may, however, be expected to result in greater ultimate stature if it causes statural growth to advance more rapidly than skeletal maturation. The present study was undertaken to find out whether, in suitable dosage, testosterone may be expected to exert a favorable influence upon the ultimate stature of short, but otherwise healthy children.

METHODS

The subjects of the study were 27 children (13 boys, 14 girls), aged 5½ to 10 years, who were of short stature for their age. Measurements of height and skeletal age were obtained for one six-month interval preceding a six-month period during which methyltestosterone was given, and for two or three six-month intervals thereafter. Random groups of 4 to 7

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children each (groups *a* through *e*) were arranged at the beginning of the study. In the second six-month period, a dose of 5, 10, 20, 30 or 40 mg. of methyltestosterone was given by mouth each day. In some of the children receiving 30 or 40 mg. per day evidence of virilism developed, and the drug was discontinued after two and a half to three months. The amount given during the entire treatment period ranged from 910 to 3,650 mg. Stature was measured as standing height, and was calculated as height age from the norms prepared by Stuart (3). Height age is the age at which the height of the individual under consideration would be the mean for a group of healthy children of the same sex. Skeletal maturation was evaluated by comparing a roentgenogram of the hand and wrist with Todd's standards (4). All measurements and interpretations of roentgenograms were made by the same person. Three separate readings of each film were made in order to minimize errors (5).

OBSERVATIONS

In the six months before treatment, the increments in height age and in skeletal age were fairly homogeneous (Fig. 1). For the group as a whole, the average height-age increment was 4.7 months (range 1 to 9 months), and the average skeletal-age increment was 4.3 months (range 0 to 12

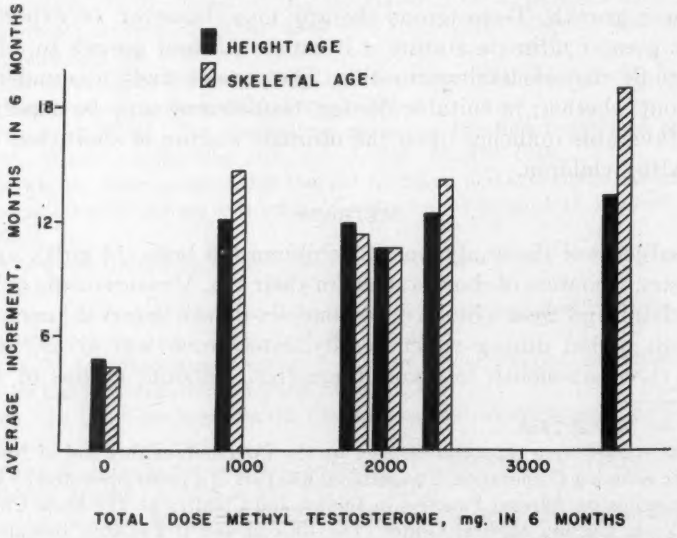

FIG. 1. Average gain in skeletal age and in height age in six months, plotted against the amount of methyltestosterone received during the same period. (Reproduced with permission from Sobel (6)).

months). Thus these children were developing more slowly than average.

When receiving testosterone, all the children experienced a distinct and rather similar acceleration in growth rate (Figs. 1 and 2), regardless of the dosage employed. The average gain in height age for the 27 children was 12.1 months in this six-month interval. In those receiving the smallest

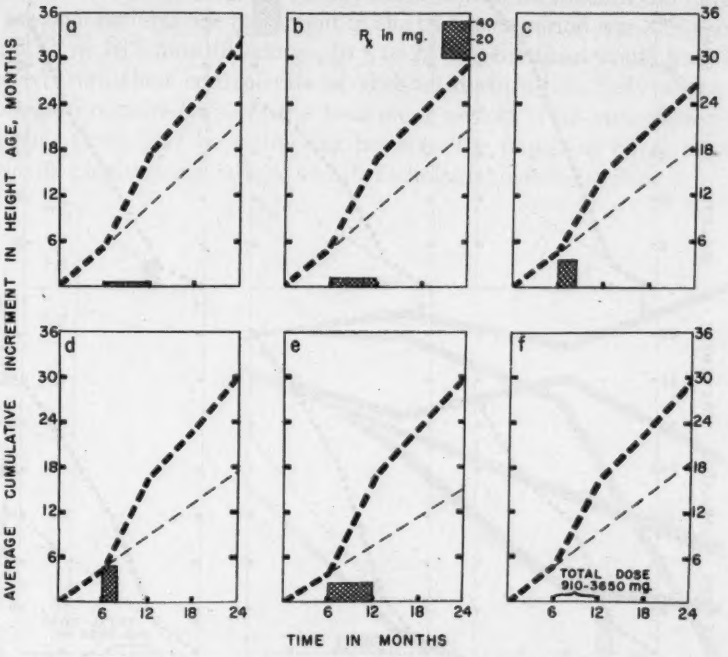

FIG. 2. Average cumulative increase in height age plotted at six-month intervals for each dosage group individually (sections *a* through *e*) and for the entire group of children (section *f*). The interrupted diagonal line in each section represents an extrapolation of the height-age gains observed in the control period. The daily dose given and the duration of treatment are shown by the hatched area in each section.

dose (5 mg. per day; 910 mg. in six months) the average increment in height age was 12.1 months in six months, whereas in those who received the largest amount (20 mg. per day; 3,650 mg. in six months) the average was 13.2 months. The average height increments of the children receiving intermediate doses were essentially alike, although in some instances a relatively small dose of testosterone was spread over the entire period (10 mg. per day; 1,830 mg. in six months), whereas in others a similar total amount was given within a shorter period (30 mg. per day for two and a

half months; 2,250 mg. in six months). In the ensuing six months the height-age increment averaged 6.5 months, with no conspicuous differences between the groups (Fig. 2). Two years after the beginning of the study (one year after the end of testosterone therapy), the average total height-age increment had been 30.0 months (Fig. 2, *f*). On the basis of

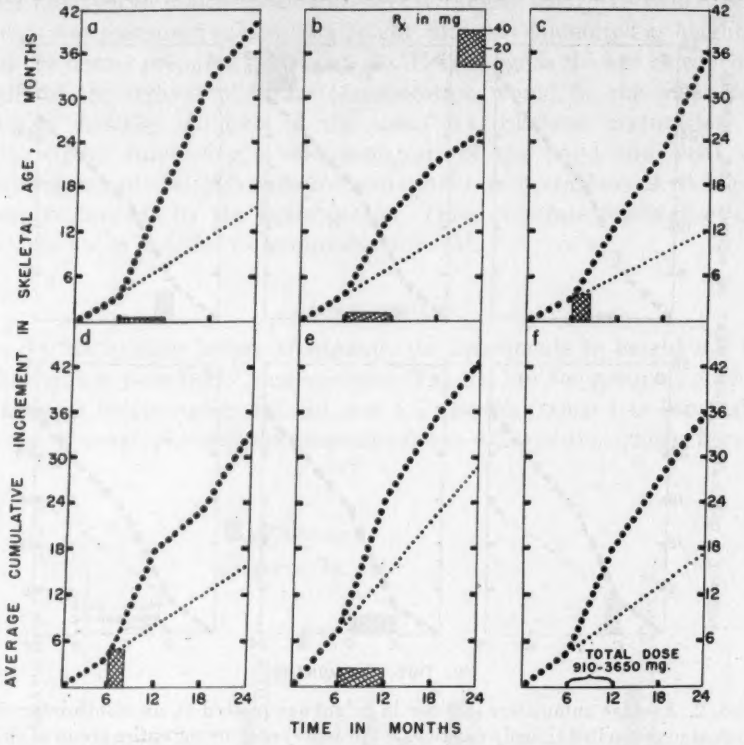

FIG. 3. Average cumulative increase in skeletal age plotted at six-month intervals for each group (section *a* through *e*) and for all 27 children (section *f*). The control rate of gain in skeletal age is indicated by the interrupted diagonal line.

average 24-month growth expectation of increase in height age in this period, there had been a gain of 6 months. But these children had been growing more slowly than average before treatment and, had there been no spontaneous change in growth rate, they would have gained only 19 months (range, 15.2 to 21.7 months) in height age during the two years. Therefore, the average increase over expected increment was 11 months. Most of this gain occurred within the period of treatment.

In each group there was a pronounced increase in skeletal maturation

following testosterone treatment (Fig. 1). The children who received 3,650 mg. showed the greatest increase: 18.6 months in six months. In the group receiving 910 mg., the average increase was 14.6 months. Since the intermediate groups showed lesser increases, the average for all 27 children was 13.9 months. Even after testosterone treatment was discontinued, there was evidence of further acceleration of skeletal maturation (Fig. 3). The average skeletal-age increment in the two-year period was 35.6 months (Fig. 3, *f*), or 18.3 months (range, 10.8 to 24.8) more than would have been expected from their control rate of skeletal maturation. Only about half of this gain occurred within the treatment period. This observation suggests that there may be a time-lag between the maturing effect of testosterone on epiphyseal cartilage and its radiologic demonstration.

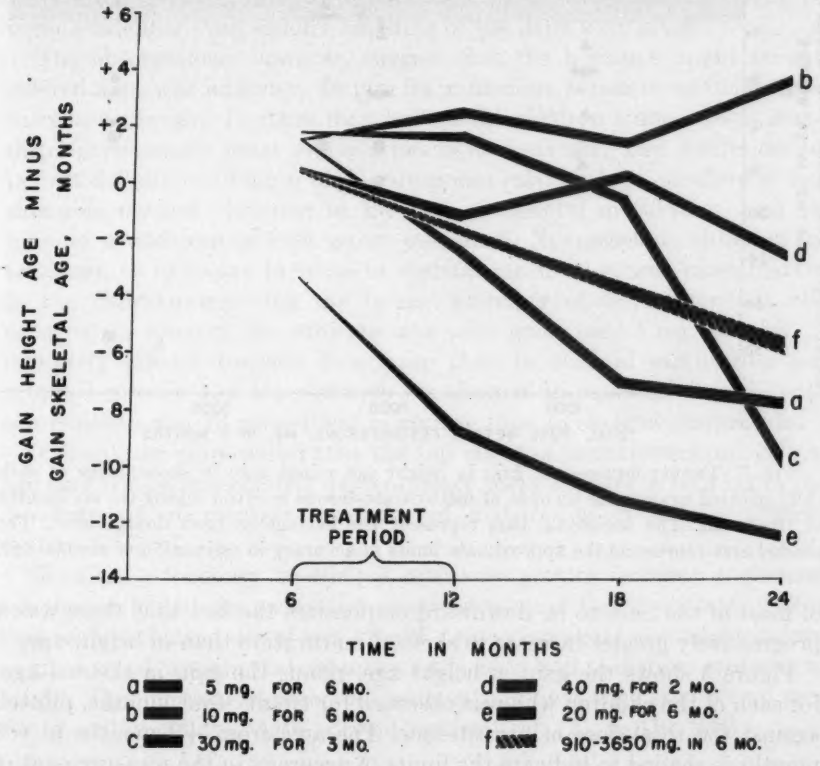

FIG. 4. Average cumulative gain in height age minus gain in skeletal age plotted at six-month intervals for each dosage group individually (*a* through *e*) and for the average of the entire group (*f*). Although in group *e* skeletal age was increasing more rapidly than height age in the control period, there was no correlation between this phenomenon and the relative degree of skeletal maturation induced by testosterone.

Thus, although the children experienced an 11-month increase in height age over that which was expected on the basis of their control rate of growth, they showed an even greater augmentation in skeletal age (18.3 months). Figure 4 shows the results obtained when the gain in skeletal age is subtracted from the gain in height age. When height age increases more than skeletal age, the value is a positive one. The tendency for the slope

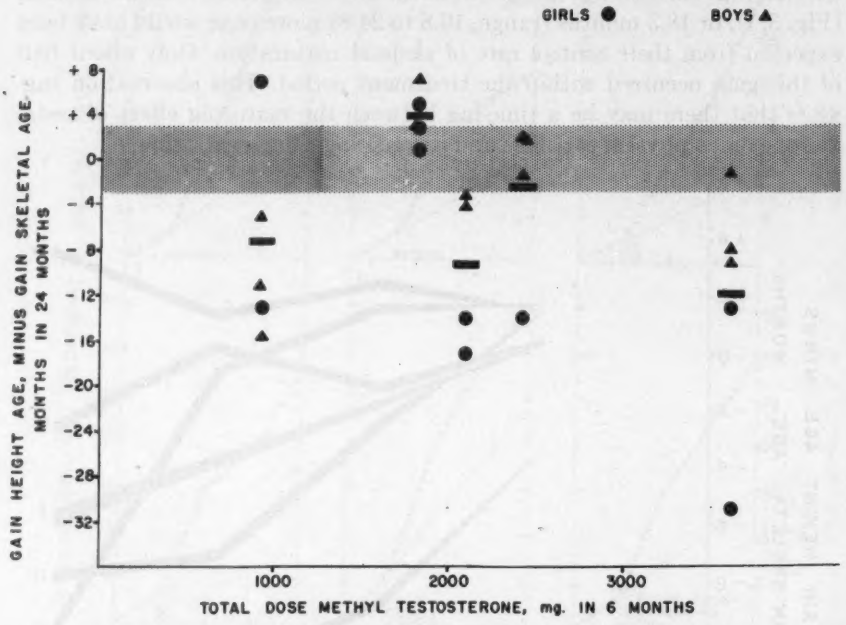

FIG. 5. Twenty-four-month gain in height age minus gain in skeletal age of each child, plotted against the amount of methyltestosterone received during the six months of treatment. The horizontal bars represent the average at each dosage level. The shaded area represents the approximate limits of accuracy in estimation of skeletal age.

of most of the lines to be downward emphasizes the fact that there was a progressively greater increase in skeletal maturation than in height age.

Figure 5 shows the gain in height age, minus the gain in skeletal age, for each of the children who was observed for twenty-four months, plotted against the total dose of testosterone. The area from +3 months to −3 months is shaded to indicate the limits of accuracy in the measurement of skeletal age. When the points fell in the shaded area, the influence of treatment on the two variables was considered to be about equal. Thus 6 of the children, or about 25 per cent of the group, gained in stature without any indication of a greater stimulus to skeletal maturation. In 4 of the

children, the gain in height predominated, although by only a small amount (height-age gain 3½ to 7 months more than skeletal-age gain in twenty-four months). In 56 per cent of the group, however, the increase in skeletal age was at least 4 months more than the increase in height age, and in some the excess increment was substantial.

DISCUSSION

It is noteworthy that so small a dose as 5 mg. of testosterone per day was as effective in promoting growth as the more usual therapeutic amounts of 10 or 20 mg. daily (1), and that still larger doses evoked no greater growth acceleration. It is possible that the daily administration of 5 mg. of methyltestosterone provides a maximal growth stimulus. A correlation between dose and magnitude of growth response might become evident if even smaller amounts of the drug were given.

The observations, however, suggest that the hormone might be considered a growth inhibitor, despite its immediate action of stimulating an increase in height. In more than half of the children studied there was a disproportionately great acceleration in skeletal age. The results do not permit definite evaluation of dose-response relationships, as there is considerable natural variation in the rate of skeletal maturation, and the number of children in each group was small. Nevertheless, although the tendency to excessive increase in skeletal maturation was most marked in the children receiving the largest amounts of testosterone, it also occurred in some of the subjects who were given only 5 mg. per day. A relatively greater increase in stature than in skeletal maturation was attained in only 4 of the children. Six showed an equal advance in both components, and 13 gained less in stature than in skeletal maturation.

It should be emphasized that the full effect of testosterone on skeletal age may not be immediately evident, since there appears to be a lag in the development of roentgen evidence of maturation of the ossification centers.

Should the tendency to elicit a relatively greater increase in skeletal age than in height age persist in repeated courses of therapy, it seems clear that with prolonged use of this growth-promoting agent one might induce so great an advance in skeletal maturation as to lead to distinct stunting in height. This outcome is observed commonly in boys with true sexual precocity and in children with adrenal virilism (ref. (2), p. 410, 234).

SUMMARY

Testosterone, which is often used as a growth hormone, stimulates both linear growth and skeletal maturation. The relative influence of oral methyltestosterone (5 to 40 mg. daily) was evaluated in 27 children, aged

5½ to 10 years, who were growing slowly. A dose of 5 mg. daily evoked as great a growth response in the six months of therapy as did larger doses. The average increase in skeletal age was greater than that in height age during the treatment period. Skeletal maturation continued to advance at greater than the control rate after therapy was concluded, whereas growth increments reverted to average normal.

In the twenty-four months of the study, bone-age and height-age increments were approximately equal in 40 per cent of the children. However, 56 per cent of them showed greater augmentation of skeletal age than of height age. This disproportionate acceleration of skeletal maturation occurred in some of the children in each dosage group. Since growth is essentially ended when epiphyseal maturation is complete, the observations suggest that testosterone may not be a suitable agent for the promotion of growth.

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TREATMENT OF EXCESSIVE GROWTH IN THE ADOLESCENT FEMALE

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GROWTH in the adolescent rarely reaches the stage of gigantism in the absence of an expanding eosinophilic adenoma of the anterior pituitary. Nevertheless excessive growth, though short of gigantism, may become an issue in the adolescent female for it is likely to raise social and economic problems capable of eliciting serious emotional disturbances. X-ray treatment or surgery, as applicable in the case of pituitary adenoma, obviously cannot be considered in the absence of a demonstrable pituitary lesion. Hence therapeutic measures are usually omitted in spite of the patient's unhappiness about her abnormal height.

It seems justified to curb such excessive growth by hormonal means if this purpose can be achieved safely. The clinical use of gonadal steroids appears to be promising, for they are known to suppress secretion of the anterior pituitary growth hormone, promote maturation of the skeleton and advance closure of the epiphyseal lines of ossification (1). Of the two steroids, estrogen is more efficient in throttling the anterior lobe of the pituitary, whereas testosterone appears to be the more powerful promoter of epiphyseal maturation (2).

The effects of gonadal steroids on skeletal growth are well illustrated by the clinical observation that in the hypogonad subject or in the sexually retarded hypopituitary dwarf the epiphyseal lines do not close at the usual age. The deficiency of gonadal steroids is common to both conditions, but in the hypopituitary dwarf growth hormone secretion is also lacking.

Application of these concepts for therapeutic purposes is no novelty. Treatment of acromegaly with estrogen has been practiced experimentally ever since estrogen became available (3), and the treatment of gigantism in the male by orchic extracts (4) preceded even the availability of testosterone preparations. The effectiveness of these steroids in gigantism is measurable by the alteration of the growth rate and eventually by demonstration of epiphyseal closure. In acromegaly, a condition in which epiphyseal ossification is completed, the direct effects on growth can be inferred only on the basis of the observed metabolic changes, *i.e.*, the decrease in

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the level of the elevated serum phosphate (5, 6). It has also been shown by animal assay (6) that testosterone treatment actually depresses growth hormone activity in the plasma.

On the basis of these considerations it was felt that the use of estrogen, with or without added testosterone, might be fruitful in obtaining arrest of growth and earlier closure of the epiphyseal lines.

CLINICAL OBSERVATIONS

In the course of years we have treated several patients along these lines. Of these, 14 remained under observation for a sufficient length of time

TABLE 1

Patient		Age (yrs.)	HEIGHT				Length of treatment (yrs.)
			Initial (inches)	Above average (inches)	Final (inches)	Total gain (inches)	
O'B	#1647	10	61½	7½	64¾	3¼	5
M.J.	#1787	9	61¼	9¼	66¾	5½	4 5/12
H.B.	#2373	14	71¼	8¼	72	¾	1/12
L.S.	#2725	12 7/12	67	9½	70	3	1 1/2
S.D.	#2796	14 7/12	68	4½	69¼	1¼	1/12
P.V.	#2980	13 8/12	66¾	4½	68⅞	2⅛	1
R.J.	#3862	13 8/12	67¾	5¼	67¾	Zero	3/12
M.S.	#4949	13 8/12	65¼	3	67	1¾	1 4/12
W.A.	#5004	16 4/12	67¾	4¼	68	¼	4/12
B.A.	#5018	13	67¼	6	68½	1¼	7/12
B.L.	#5198	12 3/12	66½	9½	67⅛	⅝	1/2
W.A.	#5199	11	69½	13	71	1½	9/12
K.E.	#5242	10 4/12	59¼	4¼	65½	6¼	3 1/12
M.E.	#5243	12	72	13	73½	1½	7/12

(Table 1). The youngest of this group was 9 and the oldest 16 years of age; the majority ranged in age from 12 to 14. The initial height varied from 59¼ to 72 inches. It was felt that treatment was indicated if: 1) the girl reached a height of 66 inches and still showed full growth potential (open epiphyseal lines), 2) the girl, though less than 66 inches in height, showed an excess of height of at least 4 inches above the average. Only once did we deviate from these rules in the case of a girl 65¼ inches tall whose height was only 3 inches above the average for her age but whose recent rapid rate of growth did not justify further waiting.

Treatment was terminated whenever the patient had not grown any more for three months and if at the same time radiologic examination of

the long bones revealed fusion of the epiphyses. The average growth increment during the period of treatment up to the described end-point was 2 inches. This figure, however, was substantially influenced by the increments in 3 patients—one who grew 5½ inches, another who grew 6¼ inches, and a third who grew 3¼ inches during treatment. These 3 patients were the youngest of the series, being 9, 10, and 10½ years of age, respectively. They were treated from three and a half to five years, and in 2 of them the treatment was intermittent and not carried out exactly according to instructions. Omitting these 3 cases, the remaining 11 showed an average growth increment of a little over 1 inch. The length of treatment for the whole group was one and half years; without the aforementioned 3 cases, the average was less than nine months.

In the earliest cases, a dose of 1.6 mg. of estradiol monobenzoate was injected every five days. Subsequently, oral medication was favored, in the form of stilbestrol at a dosage level of 2 mg. daily. Later, we changed to Premarin, 2.5 to 5.0 mg. daily; in case of uterine bleeding, this was increased to 7.5 to 10.0 mg. daily. Originally, from 5 to 10 mg. of methyltestosterone daily was added. In some of the patients the initial response to treatment appeared to be an increased rate of growth, which was particularly noticeable in those in whom the steroid combination was used. Because of this impression, we changed our approach and limited our medication to estrogen alone, at least during the first phase of the treatment; testosterone was added only after the first two or three months, when the initial growth spurt had subsided.

Height was measured every four weeks. Radiologic control consisted of examination of the epiphyses of the long bones every three months.

The medication was generally well tolerated and only one side-effect remained to be considered, *i.e.*, that on the menstrual period. Of our 14 patients, 5 had menstruated before the onset of treatment; in the others, menarche occurred in the course of treatment. In most cases, however, the menstrual cycle was suppressed during continued estrogen administration, either throughout the period of observation or at least temporarily. Whenever "break-through" bleeding occurred, the estrogen dosage was stepped up by 2.5 mg. daily. Thus we progressed from a starting dosage of 2.5 to 5.0 mg. of Premarin to a maximum dosage of 10.0 mg. per day. In 2 cases, even this high hormonal barrier was broken, with the result that there was excessive bleeding which had to be controlled by the injection of 50.0 mg. of testosterone propionate and by oral administration of ergotamine maleate. In most of our patients, however, menstruation remained suppressed during the period of treatment. When therapy was eventually terminated, the withdrawal of estrogen was preceded by the injection of 25 mg. of progesterone; thus a normal flow and subsequent regular cycles were obtained.

SUMMARY

Fourteen excessively growing adolescent girls were treated by administration of estrogen or the combination of estrogen and testosterone. After an average increment of 2 inches in height, further growth was successfully arrested. In 11 of 14 patients the average increment in height was roughly 1 inch. There was considerable variation in the length of treatment required; the general average was one and a half years but in 11 of 14 cases it was only nine months. The criteria for successful completion of treatment were 1) absence of growth for three months, and 2) radiologically determined closure of the epiphyseal lines.

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NONCONJUGATED ADRENOCORTICAL STEROIDS IN HUMAN PLASMA: COMPARISON OF THE NELSON AND SAMUELS METHOD WITH PAPER CHROMATOGRAPHIC TECHNIQUES*

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INTRODUCTION

IN RECENT years the method of Nelson and Samuels (1) for the determination of free 17-hydroxycorticosteroids (17-OH-CS) in human plasma has been used extensively. Studies of normal levels of 17-OH-CS and the effect of ACTH administration on these levels have been made (2, 3, 4). During medical or surgical stress an increase in the concentration of plasma 17-OH-CS was observed (5) and a marked rise in the levels prior to death was reported by Sandberg *et al.* (6). Studies of 17-OH-CS levels in schizophrenic patients under various conditions have also been carried out (7, 8). It should be noted that the Nelson and Samuels method does not measure the corticoid conjugates which have been detected recently in human blood (9).

Cortisol (compound F) and, in smaller quantities, corticosterone, were isolated from human adrenal venous blood by Romanoff *et al.* (10). Cortisol is known to be the corticoid present in largest amount in the blood plasma of man and its concentration is increased following the administration of ACTH (11). There is no direct proof, however, that the increased free 17-OH-CS levels found in various conditions by the method of Nelson and Samuels are due to a change in cortisol. It is the purpose of the present paper to compare the results obtained by the Nelson and Samuels method with those found by chromatographic techniques which permit separation of cortisol.

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MATERIAL AND METHODS

Experimental subjects: Blood was drawn from 6 normal, resting subjects and 6 subjects under severe medical or surgical stress. Patients with chronic schizophrenia who had been hospitalized in an institution for a period of at least five years were investigated. Blood samples were also collected from patients just prior to death.

Methods: Samples of blood measuring 40–80 ml. were collected into heparinized tubes (0.8 mg. of heparin per 40 ml. of blood). The tubes were centrifuged and the plasma was separated from the red cells. Plasmas which were not extracted immediately were stored in a deep-freeze.

An aliquot (10 ml.) of the plasma samples was used for the measurement of 17-OH-CS by the method of Nelson and Samuels (1) as modified by Eik-Nes *et al.* (12).

The remainder of the plasma was extracted three times with twice its volume of freshly redistilled chloroform. The pooled chloroform extracts were evaporated to dryness in a water bath at 40° C., using a current of nitrogen.

The extract was then dissolved in 20 ml. of a Skellysolve C-ethyl acetate mixture (1:1 by volume). This solution was passed through 2 Gm. of silica gel (Davidson, 60 ×200 mesh) in a 1-cm. diameter column, as described by Bush and Sandberg (11). The steroids were eluted with 7 ml. of a methanol-ethyl acetate mixture (1:1 by volume), and the eluate was evaporated to dryness at 40° C. under a current of nitrogen.

The dry extract was then dissolved in a small amount of methanol:chloroform (1:1 by volume) and transferred to a strip of Whatman No. 2 filter paper. The extract was concentrated on the origin with ethyl acetate:methanol (2:1), according to the method of Bush (13). The paper was placed in a chromatography tank containing the system benzene/55% methanol in water, equilibrated overnight and then chromatographed for approximately four hours. Reference steroids were chromatographed alongside the extracts.

Following chromatography, the paper strips were examined with the ultraviolet (UV) scanner (14, 15); the area corresponding to the authentic standard compound F was delimited, cut out, and eluted with ethanol. The ethanol extract was evaporated to dryness.

The dry residue was dissolved in 0.5 ml. of twice redistilled methanol. An aliquot of 0.2 ml. was transferred into each of two test tubes. To one of the tubes, 0.3 ml. of Nelson-Samuels (1) reagent (16 mg. of phenylhydrazine hydrochloride per 10 ml. of 75 per cent H_2SO_4) was added. To the second test tube, which served as a blank, 0.3 ml. of 75 per cent H_2SO_4 was added. After mixing, the tubes were incubated at 60° C. for sixty minutes. The derived optical density of the samples was determined as described by Nelson and Samuels (1).

In most samples, spots with R_F values different from those of cortisol, cortisone, corticosterone or Reichstein's compound S were also found on the UV scanner. In certain cases such spots were eluted from the paper and a Porter-Silber reaction was carried out on the eluate. In other cases the paper chromatogram, except for the area corresponding to cortisol, was sprayed with 1 N NaOH and an 0.08 per cent ethanol solution of blue tetrazolium (BTZ); the strip was then allowed to stand in the dark at room temperature until dry. On some specimens the combined NaOH-triphenyltetrazolium chloride (TPTZ) test (13) was carried out instead of the BTZ test.

In this paper the term 17-OH-CS refers to levels measured by the method of Nelson and Samuels (1) and the term cortisol (compound F) to levels determined by the paper chromatographic technique.

RESULTS

Recoveries of pure steroids

In order to test the reliability of the methods used in this study recoveries of cortisol, cortisone, and Reichstein's substance S were measured. The recoveries of 5 and 10 μg. of these steroids following chromatography on a silica-gel column were 90–94 per cent, and those following paper chromatography were 82–91 per cent. The recoveries through the combined column and paper chromatographies were 72–81 per cent. Similar

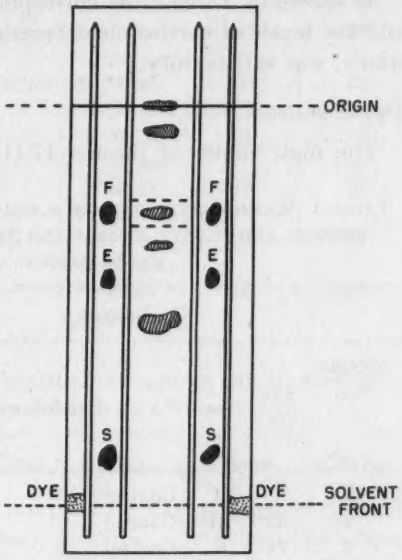

FIG. 1. *Paper chromatogram of plasma extract from normal subjects (sample No. 3) as it appeared on UV scanner.* Reference steroids were chromatographed on each side strip of paper and the plasma extract on the center strip. The area of the plasma extract strip corresponding to compound F, as outlined by the broken line, was eluted and the Porter-Silber reaction carried out on the eluate. In addition to the spot corresponding to compound F, 2 other UV-absorbing spots (X_1 and X_3) were almost always found, and a third one (X_L) occasionally. These spots never showed a positive color reaction with TPTZ or BTZ.

results were obtained when the pure steroids were added to plasma and carried through the entire procedure.

Cortisol recoveries were made with each set of determinations, and the results obtained for plasma were corrected in the same manner in which the Nelson and Samuels method applies a correction for the recovery of cortisol through that procedure.

Normal individuals

The results are shown in Table 1. Considering the minute amounts of material being measured, the complexity of both procedures, and the inability to run duplicates on each determination, Cases 2, 3 and 4 showed very good correspondence between the 17-OH-CS value measured by the Nelson and Samuels method and the compound F value as determined by

the chromatographic technique. Plasma extracts from Cases 5 and 6 were pooled for paper chromatography and the concentration of compound F was somewhat lower than the average for 17-OH-CS.

Medical and surgical stress

The results obtained by the two methods (Table 1) were satisfactorily similar, except for some discrepancy in Cases 9 and 12.

Chronic schizophrenia

As shown in Table 2 the correspondence between the 17-OH-CS values and the levels of cortisol as determined by paper chromatography, on the whole, was satisfactory.

Dying patients

The high values of plasma 17-OH-CS found just prior to death cor-

TABLE 1. MEASUREMENT OF FREE PLASMA 17-OH-CS BY A PAPER CHROMATOGRAPHIC METHOD, AND THAT OF NELSON AND SAMUELS, IN NORMAL SUBJECTS AND THOSE UNDER MEDICAL OR SURGICAL STRESS

Sample No.	Subject			Time of plasma collection	Plasma 17-OH-CS (μg./100 ml.) Nelson and Samuels	Plasma Compound F (μg./100 ml.) Paper chromatography
	Age (yrs.)	Sex	Condition			
1		M	Control	8:10 A.M.	—	11
2	32	M	Control	8:00 A.M.	14	14
3	24	F	Control	11:00 A.M.	17	15
4	29	M	Control	11:00 A.M.	12	11
5	26	M	Control	11:00 A.M.	18	8
6	23	M	Control	11:00 A.M.	11	
7	40	F	Acute pneumonia; very ill	1:00 P.M.	17	20
8	35	F	Cirrhosis of liver; bleeding	2:00 P.M.	25	22
9	38	F	Cirrhosis of liver; vena cava-portal shunt; operation lasted 8½ hrs.	4:00 P.M.	20	43
10	50	F	Cholecystectomy; operation lasted 4 hrs.	12:00 Noon	33	29
11	70	M	Cholecystectomy; operation lasted 3 hrs.	2:00 P.M.	33	26
12	65	F	Cholecystectomy; operation lasted 2½ hrs.	11:30 A.M.	62	40

TABLE 2. MEASUREMENT OF FREE PLASMA 17-OH-CS BY A PAPER CHROMATOGRAPHIC METHOD AND THAT OF NELSON AND SAMUELS, IN SCHIZOPHRENIC PATIENTS
(In each case samples from several patients were pooled)

Sample No.	Sex of patient	Conditions of measurement	Time of plasma collection	Plasma 17-OH-CS (μg./100 ml.) Nelson and Samuels	Plasma Compound F (μg./100 ml.) Paper chromatography
13	M	No treatment	8:00 A.M.	14	8
14	F	No treatment	10:00 A.M.	8	8
15	F	Two hrs. following injection of 30 mg. ACTH I.V.	10:00 to 10:30 A.M.	32	30
16	M	Two hrs. following injection of 30 mg. ACTH I.V.	10:00 to 10:30 A.M.	30	22
17	F	During insulin coma	9:30 to 10:30 A.M.	21	16
18	M	During insulin coma	9:30 to 10:30 A.M.	19	20
19	M	During insulin coma	9:30 to 10:30 A.M.	24	17
20	M	Following electroshock	9:00 to	39	32
	F	Following electroshock	10:00 A.M.		

TABLE 3. MEASUREMENT OF FREE PLASMA 17-OH-CS BY A PAPER CHROMATOGRAPHIC METHOD AND THAT OF NELSON AND SAMUELS, IN DYING PATIENTS

Sample No.	Patient			Time of plasma collection		Plasma 17-OH-CS (μg./100 ml.) Nelson and Samuels	Plasma Compound F (μg./100 ml.) Paper chromatography
	Sex	Age (yrs.)	Cause of death	Hrs. before death	Time of day		
21	M			12 hrs.	11 A.M.	—	21
22	M	80	Pneumonia	25 min.	11 P.M.	52	22
23	M	84	Uremia	15 min.	6:20 P.M.	50	32
24	M	60	Cirrhosis, of liver; lymphosarcoma	26 hrs.	1:30 P.M.	20	20
25	M	80	Cerebral vascular accident	0	12:15 P.M.	35	32
26	M	18	Aplastic anemia	2 min.	9:00 P.M.	48	48
27	F	77	Acute pyelonephritis	1 hr.	8:00 A.M.	32	36
28	F	64	Acute leukemia	0	4:30 P.M.	47	48

responded to an increase in cortisol concentration as determined by paper chromatography (Table 3). There was good correspondence in all except Cases 22 and 23.

Compounds other than cortisol

In addition to cortisol, other spots absorbing the ultraviolet light at 250 mμ were found quite regularly (Table 4). One was near the origin ($R_F = 0.050$) and a second was slightly less polar than compound E ($R_F = 0.515$). A third

TABLE 4. INCIDENCE OF SPOTS WHICH ABSORB UV LIGHT BUT DO NOT REACT IN TPTZ AND BTZ TESTS ON VARIOUS PAPER CHROMATOGRAMS. THE NUMBER OF PLUS SIGNS INDICATES THE NUMBER OF SPOTS FOUND IN THE REGION OF THE R_F SPECIFIED

Sample No.	Conditions of measurement	Spot $R_F = 0.050$	Spot $R_F = 0.310$	Spot $R_F = 0.515$
1	Normal control	+	+	+
2	Normal control	++	0	+
3	Normal control	+	+	+
4	Normal control	+	0	+
5–6	Normal control	+	0	+
7	Medical stress	+	+	+
8	Medical stress	+	0	+
9	Surgical stress	+++	+	+
10	Surgical stress	0	0	+
11	Surgical stress	+	0	0
12	Surgical stress	+	0	0
13	Schizophrenia	+	0	+
14	Schizophrenia	+	+	+
15	Schizophrenia; ACTH	+	0	+
16	Schizophrenia; ACTH	+	+	+
17	Schizophrenia; insulin	+	0	+
18	Schizophrenia; insulin	+	0	0
19	Schizophrenia; insulin	+	0	+
20	Schizophrenia; electroshock	++	+	+
21	Dying patient	++	0	+
22	Dying patient	0	0	0
23	Dying patient	0	0	+
24	Dying patient	+	+	+
25	Dying patient	++	0	0
26	Dying patient	+	0	+
27	Dying patient	+	+	+
28	Dying patient	+	0	+

spot with an R_F of 0.310 was sometimes present (Table 4). On occasions, two spots instead of one were found near the origin.

In certain cases these spots were eluted from the paper strip and a Porter-Silber reaction was carried out on the eluate. The results were always negative. In the other cases the paper strips were sprayed with 2 N NaOH and BTZ or TPTZ. The various spots described in the previous paragraph did not show a color reaction with these tests.

DISCUSSION

In the various conditions studied, plasma 17-OH-CS levels as measured by the Nelson and Samuels method showed reasonably good agreement with the corresponding levels of cortisol as determined by paper chromatography. It must be recognized that in most of these analyses the investigator was depending on the determination of 1–3γ of steroid separated from 10 ml. of plasma. The colorimetric procedures were being used at the limits of their sensitivity. In addition, any error of manipulation (such as failure to dissolve a residue completely) could not be identified, since it was impossible to run duplicate analyses by each method with the amounts of blood available. These results, therefore, justify the use of the Nelson and Samuels method in such conditions and indicate that the method does, in fact, measure preponderantly 17-OH-CS in plasma.

In the present study corticosterone was not detected on paper chromatograms on any occasion. Bush and Sandberg (11), however, were able to detect it in a few extracts of human plasma and the ratio cortisol-corticosterone was shown to be 5–10:1. These authors used plasma volumes five to ten times larger than those used in the present study, and it is probable that corticosterone was present in quantities too small to be detected on our paper chromatograms. Morris (16) and Sweat *et al.* (17) found large amounts of corticosterone in human plasma. The possible causes for such a disagreement have been discussed elsewhere (11).

As previously shown for plasma 17-OH-CS (5), there was a definite increase of cortisol concentration during medical and surgical stress.

No difference in pattern was found between the paper chromatograms of normal subjects and those of patients with chronic schizophrenia. In such patients ACTH administration, insulin coma and electroshock therapy induced an increase in cortisol concentration comparable to that of normal subjects.

The level of free 17-OH-CS in the plasma of dying patients has been reported to be elevated. The present study showed that in most cases it was due to an increase of cortisol, as determined by paper chromatography.

The significance of spots which absorb ultraviolet light, but react nega-

tively to other tests (BTZ, TPTZ), is not clear. Further work is needed to determine the structure of the compounds causing such spots on paper chromatograms.

SUMMARY

The results obtained by the Nelson and Samuels method for the measurement of free 17-hydroxycorticosteroids in human plasma were compared with those obtained by the measurement of cortisol as separated by a paper chromatographic technique.

In normal controls, in patients under surgical and medical stress, in schizophrenic individuals and in dying patients, there was in most cases a satisfactory agreement between the results obtained by the two methods.

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BLOOD ADRENOCORTICOTROPIN (ACTH) IN CHILDREN WITH RHEUMATIC FEVER*

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THE concentration of adrenocorticotropic hormone (ACTH) in the circulating blood has been measured in normal human subjects and in individuals with a few disease entities. Paris *et al.* (1) recently reviewed the literature concerning ACTH in the blood of human subjects, as determined by adrenal ascorbic-acid depletion assay technics. They attribute most of the wide variations reported for ACTH concentration to differences in the blood extraction technics. The oxycellulose extraction method (2) yields results compatible with those found when plasma alone is assayed for its ACTH content (1). Neither of these procedures has sufficient sensitivity to detect the concentration of blood ACTH in most normal human subjects. However, they are capable of detecting the elevated concentrations which occur in patients with certain pathologic conditions.

In a previous report (3) there was a suggestion that endogenous blood ACTH concentrations might be elevated in patients with rheumatic fever. In such patients, abnormal 17-hydroxycorticosteroid (17-OHCS) blood concentrations are also found. Elevated concentrations occur during the "early acute" phase of the disease, and abnormally low concentrations occur in the period of "well-established" active rheumatic fever, as well as in chorea or in rheumatic fever which is inactive (4). In view of these altered 17-OHCS concentrations, and the suggestion of elevation of endogenous blood ACTH in rheumatic fever, it seemed of interest to extend the observations concerning blood ACTH levels in various phases of this disease.

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MATERIALS AND METHODS

The subjects of this study were 32 children with rheumatic fever, ranging in age from 7 to 18 years. In addition, there were 7 patients with "pure" chorea. The diagnosis of rheumatic fever was made in accordance with the criteria proposed by Jones (5) as modified by Denny and his associates (6). Blood samples (120–150 ml.) were obtained for ACTH assay by venipuncture and immediately placed in 4 volumes of glacial acetic acid. These specimens were refrigerated until the blood-glacial acetic acid mixtures were processed by the oxycellulose extraction technic (2) and the resulting blood eluates were assayed in male hypophysectomized rats by the adrenal ascorbic-acid depletion method (7). In most instances, each of 3 rats received the extract from 40 ml. of blood in an 8-ml. volume.

On each day that samples were assayed, freshly prepared ACTH standard solutions containing 0.25, 0.5, and 1.0 milliunit[1] per 100-gram assay rat were injected, in order to obtain the calibration curve for the given day. In the tables, the adrenal ascorbic-acid depletion caused by the blood eluates as well as by the 3 doses of standard assayed on the corresponding day are presented. Due to the difficulty of comparing adrenal ascorbic-acid depletion values from day to day, an attempt was made to express these values as milliunits of ACTH per liter (mU/L). This form of expression permitted the direct comparison of values from an assay on one day with those from an assay on another day. This conversion was accomplished in the following manner: The calculated regression line was used as the standard curve for the day. Using this line and the observed adrenal ascorbic-acid depletion[2] (AAD), the ACTH concentration in the unknown sample was calculated graphically. This calculation can be expected to yield no better than a rough approximation of the true ACTH concentration value.

Since the response to saline on a given day was variable, and the mean AAD varied approximately from +10 to −10 on different days, it was decided arbitrarily, for the sake of conservatism, to consider a mean of less than 15 (AAD) to be in the range of saline response, thus indicating no detectable ACTH in the sample assayed.

OBSERVATIONS

Table 1 shows the results of 26 ACTH assays on the blood of children with untreated active rheumatic fever. In normal children ACTH concentrations are undetectable by this method (3). In this table, as in succeeding tables, the mean AAD value for the blood eluate of the unknown sample, and the mean AAD value derived from the line of best fit for the doses of standard ACTH employed, are presented. In addition, the lambda value[3] for the standard assay of the day on which the given sample was processed is listed. The *approximate* value for the concentration of ACTH, derived by the previously described method, is shown in the last column of the table.

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[1] One milliunit equals one one-thousandth of a U.S.P. unit of ACTH, which is defined as the activity of 1 milligram of the International Standard ACTH (La-1-A).

[2] AAD is expressed as the difference in ascorbic-acid concentration (mg./100 Gm. fresh adrenal) between the left adrenal, removed prior to injection of test material, and the right adrenal, removed one hour after the injection of test material.

[3] Lambda = an index of the inherent precision of the assay (7).

TABLE 1. BLOOD ACTH CONCENTRATIONS IN PATIENTS WITH ACTIVE RHEUMATIC FEVER

Patient	Duration of symptoms	Assay standardization				Blood sample	
		Mean AAD responses to standard ACTH			Lambda	Mean AAD	ACTH (mU/L)
		.25 mU	.5 mU	1.0 mU			
G.S.	3 days	49.4	69.5	89.6	.585	17	0
E.R.	3 days	6.6	88.0	169.4	.07	0	0
L.L.	4 days	6.6	88.0	169.4	.07	0	0
C.C.	4 days	2.4	81.5	160.6	.114	2	0
D.W.	7 days	−8.2	40.6	89.4	.193	0	0
J.C.	11 days	65.1	109.7	154.4	.182	37	8.6
N.H.	11 days	5.0	70.9	136.8	.09	136	25.0
C.W.	12 days	17.8	—	100.1	.20	0	0
D.S.	12 days	17.8	—	100.1	.20	91	21.4
I.S.	14 days	5.0	70.9	136.8	.09	125	22.0
D.R.	14 days	34.8	100.4	166.0	.221	24	6.8
B.S.	15 days	41.5	—	104.8	.197	37	5.7
S.B.	15 days	5.0	70.9	136.8	.09	70	12.5
J.M.	16 days	6.6	88.0	169.4	.07	31	7.5
A.D.	3 wks.	5.0	70.9	136.8	.09	16	7.0
K.H.	3 wks.	26.0	67.2	108.4	.185	12	0
S.D.	3 wks.	5.0	70.9	136.8	.09	31	8.2
S.S.	4 wks.	65.1	109.7	154.4	.182	207	34.2
L.A.	4 wks.	35.3	106.8	158.8	.111	35	5.8
M.P.	6 wks.	−8.2	40.6	89.4	.193	27	10.3
J.B.	8 wks.	96.7	119.1	141.5	.350	145	28.0
T.D.	8 wks.	65.1	109.7	154.4	.182	30	4.1
M.P.	12 wks.	21.3	78.4	135.6	.204	26	6.6
P.E.	12 wks.	34.8	100.4	166.0	.221	86	9.6
T.F.	17 wks.	34.8	100.4	166.0	.221	151	9.5
D.S.	25 wks.	49.4	69.5	89.6	.585	126	87.8

In this group of untreated patients with active rheumatic fever there was observed a relationship between duration of rheumatic activity and concentration of ACTH in the blood. None of the 5 patients in whom the duration of symptomatology was less than seven days had a sufficient concentration of blood ACTH to be detected by the technic employed. In the blood of patients who had been ill more than seven days, 19 of 21 determinations revealed measurable ACTH levels. Among these patients, who had been ill from eleven days to twenty-five weeks, there was no evidence of a trend indicating that the blood levels either increased or decreased with the duration of illness. Although some of these children had

considerably higher blood levels of ACTH than others in the series, there were no clinical findings wihch distinguished them from the remainder of the group.

Table 2 shows data concerning ACTH levels in the blood of 7 patients with Sydenham's chorea. Only inconsistent elevations of blood ACTH concentrations were observed in this group, 3 of the 7 children having elevated levels. An additional patient, P.A., had an elevated ACTH concentration, as calculated from the regression curve for the day. However,

TABLE 2. BLOOD ACTH CONCENTRATIONS IN PATIENTS WITH CHOREA

Patient	Assay standardization				Blood sample	
	Mean AAD responses to standard ACTH			Lambda	Mean AAD	ACTH (mU/L)
	.25 mU	.5 mU	1.0 mU			
L.H.	79.5	120.3	151	.222	66	4.3
D.J.	79.5	120.3	151	.222	20	0
C.E.	22.1	68.8	115.6	.116	9	0
C.E.	33.5	108.9	184.3	.09	24	5.7
P.A.	22.1	68.8	115.6	.116	14	0*
M.L.	2.4	81.5	160.6	.114	17	6.0
B.P.	33.5	108.9	184.3	.09	0	0

* As calculated from the standard regression curve, this value is 6.0. Since AAD 15 is considered to be in the saline range, this value becomes zero.

the mean AAD for this assay was 14, which falls within the limits of the range arbitratily attributed to saline; therefore, for purposes of this report, this value was considered as indicating no detectable ACTH.

Table 3 shows the results of 17 determinations of blood ACTH in 9 different patients with inactive rheumatic fever. Blood samples were obtained from ten weeks to two and a half years after cessation of all therapy and after the last evidences of rheumatic activity. In 15 of the 17 samples there were elevated concentrations of ACTH. One sample in each of 2 patients showed no detectable ACTH; in each of these 2 patients duplicate samples for assay had been obtained within a week. Each patient whose blood ACTH level was zero on one determination had a definitely elevated concentration of ACTH on the other determination, although in neither case was there any detectable change in the patient's clinical status in the interim between obtaining the blood samples.

Data concerning the few available ACTH assays on the blood of rheumatic fever patients, during and immediately after cortisone treat-

TABLE 3. BLOOD ACTH IN PATIENTS WITH INACTIVE RHEUMATIC FEVER

Patient	Sex	Time since activity or therapy	Assay standardisation				Therapy	Blood sample	
			Mean AAD responses to standard ACTH			Lambda		Mean AAD	ACTH (mU/L)
			.25 mU	.5 mU	1.0 mU				
L.A.	M	10 wks.	46.3	115.9	185.5	.106	Cortisone	13	0
T.F.	M	10 wks.	26.0	67.2	108.4	.185	Cortisone	87	17.4
L.A.	M	11 wks.	41.5	—	104.8	.197	Cortisone	23	4.2
T.F.	M	12 wks.	21.3	78.4	135.6	.204	Cortisone	41	8.0
D.S.	M	12 wks.	41.5	—	104.8	.197	Cortisone	24	4.2
T.F.	M	14 wks.	−8.2	40.6	89.4	.193	Cortisone	22	9.6
J.M.	M	13 mos.	41.5	—	104.8	.197	ACTH	24	4.2
J.M.	M	13 mos.*	41.5	—	104.8	.197	ACTH	26	4.4
W.K.	M	13 mos.	41.5	—	104.8	.197	Cortisone	30	4.8
W.K.	M	13 mos.*	41.5	—	104.8	.197	Cortisone	19	3.8
J.F.	M	28 mos.	21.3	78.4	135.6	.204	Bed rest	0	0
J.F.	M	28 mos.*	21.3	78.4	135.6	.204	Bed rest	41	8.0
M.F.	M	28 mos.	21.3	78.4	135.6	.204	ACTH	28	6.8
M.F.	M	28 mos.*	21.3	78.4	135.6	.204	ACTH	25	6.5
M.F.	M	28½ mos.	21.3	78.4	135.6	.204	ACTH	31	7.0
C.J.	F	30 mos.	21.3	78.4	135.6	.204	ACTH	89	14.2
S.G.	M	**	21.3	78.4	135.6	.204	None	30	7.0

* Plus one week.

** Progressive cardiac involvement for several years without diagnosable rheumatic attack.

TABLE 4. INFLUENCE OF CORTISONE THERAPY ON BLOOD ACTH CONCENTRATIONS IN PATIENTS WITH RHEUMATIC FEVER

Patient	Assay standardization				Day of therapy	Blood sample	
	Mean AAD responses to standard ACTH			Lambda		Mean AAD	ACTH (mU/L)
	.25 mU	.5 mU	1.0 mU				
S.B.	5.0	70.9	136.8	.09	3	17	7.1
J.B.	49.4	69.5	89.6	.585	10	0	0
					Days after therapy		
L.A.	65.1	109.7	154.4	.182	1	6	0
T.F.	34.8	100.4	166.0	.221	1	6	0
A.D.	26.0	67.2	108.4	.185	2	32	6.9
C.C.	2.4	81.5	160.6	.114	3	26	7.7
A.W.	79.5	120.3	161.1	.222	4	0	0
T.L.	35.3	106.8	158.8	.111	8	26	5.2
L.A.	65.1	109.7	154.4	.182	8	21	2.9

ment, are presented in Table 4. A single determination on the third day of treatment in one patient revealed an elevated value, whereas a determination on the tenth day of treatment in another patient showed no detectable ACTH. In the post-therapy period, 2 samples on the first day showed zero values, whereas, with one exception, those obtained between the second and eighth days after therapy showed elevated ACTH concentrations. The values during and after therapy are compared with the pre-therapy values for individual patients in Table 5. The data in Tables 4 and 5, although not extensive enough to be conclusive, suggest that within

TABLE 5. COMPARISON OF BLOOD ACTH CONCENTRATIONS IN DIFFERENT PHASES OF THERAPY IN RHEUMATIC FEVER PATIENTS TREATED WITH CORTISONE

Patient	Pre-therapy	During therapy		After therapy	
		Day of therapy	ACTH (mU/L)	Days after therapy	ACTH (mU/L)
S.B.	12.5	3	7.1		
J.B.	28.0	10	0		
L.A.	5.8			1	0
				8	2.9
T.F.	9.5			1	0
A.D.	7.0			2	6.9
C.C.	0			3	7.7

a few days after initiation of cortisone therapy in these patients the ACTH concentrations in the blood became depressed and remained so until after cessation of cortisone, when they again became elevated.

DISCUSSION

The data presented here indicate that in patients with rheumatic fever the blood ACTH concentration is related to the phase of rheumatic activity. Even though the values reported may not represent precise determinations of the actual concentrations of circulating ACTH, the results are consistent and the pattern of change demonstrated throughout the course of illness with rheumatic fever is distinct. In children with "early acute" rheumatic fever (*i.e.*, during the first week after the onset of rheumatic symptomatology), as in normal children (3), circulating blood concentrations of ACTH are not detectable by the method employed. However, in patients with "well-established" active rheumatic fever, inactive rheumatic fever, or chorea these concentrations are elevated. Conversely, as previously reported (4), the plasma concentrations of 17-OHCS are higher than normal in patients with "early acute" rheumatic

fever and lower than normal in patients with "well-established" active or inactive rheumatic fever or with chorea.

Little is known about the mechanisms by which elevations of blood endogenous ACTH concentration take place, as the factors influencing the rate of ACTH secretion and its fate thereafter remain obscure. The postulation that ACTH secretion by the pituitary is regulated, either directly or indirectly, by the concentration of circulating corticosteroids is supported by certain experimental evidence. According to this concept, a decrease in the circulating corticosteroid level would cause an increased rate of release of ACTH by the pituitary; and an increase in the blood corticosteroid level would result in a decreased rate of release of ACTH. In animals (8, 9), adrenalectomy results in elevated blood ACTH concentrations. Similarly, elevations of circulating ACTH have been observed in patients with Addison's disease (10–12) and with congenital adrenal hyperplasia (3), two conditions in which blood corticosteroid levels are low. In addition, it has been demonstrated that cortisone therapy causes the elevated ACTH concentration in patients with congenital adrenal hyperplasia to decrease toward normal (3). Although the pertinent data in the present report are few (Tables 4 and 5), there is a suggestion that cortisone therapy likewise exerts a suppressive effect upon circulating ACTH concentrations in patients with rheumatic fever. On the other hand, the data of Sydnor *et al.* (10), who recently reported elevated blood ACTH levels in 3 Addisonian patients despite cortisone therapy, are difficult to explain on the basis of this concept.

The observations of an inverse relationship between blood ACTH and 17-OHCS concentrations in patients with untreated rheumatic fever are in conformity with this concept of the humoral control of ACTH secretion. The elevated ACTH levels in these patients may be considered secondary to the decreased corticosteroid levels. The latter might be attributable to adrenocortical hypofunction or to an accelerated rate of removal of steroids from the circulation. Available data pertinent to this question include observations concerning urinary steroid excretion, which is not increased in these patients (13), and the half-life of exogenous hydrocortisone, which is longer than normal in patients with active rheumatic fever (14). Although the latter observation does not eliminate the possibility of an increased rate of tissue utilization of adrenal steroids, it indicates that the low plasma steroid concentrations are not attributable to an increased rate of their removal from the circulation. These data suggest that in patients with rheumatic fever the low corticosteroid concentrations in the blood are due to adrenal hypofunction.

This adrenal hypofunction may be "segmental," with decreased produc-

tion of 17-hydroxycorticosteroids but not of other types of adrenal hormones. There are no data available which bear directly on this question. Also, the low circulating steroid levels in these patients in the presence of elevated ACTH levels may reflect an elevated adrenal threshold to ACTH stimulation. In this regard, it is known that rheumatic fever patients have normal 17-OHCS responses to the administration of relatively large doses of ACTH (25 I.U. intramuscularly) (15).

The elevated blood ACTH levels in these patients conceivably could be due to abnormal pituitary function. Since ACTH concentrations were not elevated during the initial phase of rheumatic fever, primary pituitary hypersecretion of ACTH appears unlikely; also, corticosteroid concentrations would be expected to be elevated, but were found to be depressed, when the ACTH concentrations were elevated. The formation (either initially by the pituitary, or subsequently by an altered metabolism) of an abnormal type of ACTH, with an impaired ability to stimulate steroidogenesis, but with an unimpaired adrenal ascorbic-acid depleting activity, seems possible but lacks substantiation. Moreover, the possibility should be considered that ACTH is utilized or altered in the process of steroidogenesis and therefore has a longer half-life in the subject with adrenal insufficiency than in the normal subject. In this regard, the half-life of endogenous ACTH has been determined only in the adrenalectomized animal (16) and the corresponding value in the intact animal remains unknown. The concept of "active" and "activable" ACTH which recently has been proposed (17) is interesting, but needs confirmation before it can be considered seriously in interpreting the data presented here.

The occurrence of elevated 17-OHCS and undetectable ACTH concentrations during the "early acute" phase of rheumatic fever may seem somewhat paradoxical. During this period of acute illness one might expect the increased plasma 17-OHCS levels to reflect an increased secretion of ACTH; yet the levels of circulating ACTH were not measurable. Apparently this pattern is consistent with that existing in patients with other acute illnesses. Such patients have elevated 17-OHCS concentrations (15, 18, 19); however, no elevations of circulating ACTH, sufficient to be detected by the technic employed here, have been observed in the few acutely ill patients studied (3, 10, 20). Possibly an increased secretion of ACTH does occur temporarily, thereby effecting an increased concentration of circulating corticosteroids. These concentrations in turn may inhibit for a prolonged period any further secretion, so that a pattern of elevated steroid and undetectable ACTH concentrations results. Present evidence suggests that the *in vivo* half-life of endogenous ACTH is not greater than a few minutes (16); on the other hand, the half-life of corti-

costeroids normally is much longer (14, 21, 22), and in acute rheumatic fever is prolonged even further (14). This relationship between the half-life of ACTH and that of corticosteroids is compatible with such a concept.

Several possible explanations of the ACTH-steroid patterns observed in these patients with rheumatic fever have been presented. At the present time available data do not permit a definitive interpretation.

SUMMARY

Data are presented concerning the concentration of circulating ACTH in patients with various phases of rheumatic fever. During the "early acute" phase, *i.e.*, the first seven days after onset of symptomatology, these concentrations are undetectable, as they are in normal children. In "well-established active" rheumatic fever, inactive rheumatic fever, and chorea, the blood levels of ACTH are elevated. Data are presented which suggest that cortisone therapy depresses blood ACTH levels during the period of its administration, but that there is a return to elevated values soon after the discontinuation of cortisone. Some implications of these findings are discussed.

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Teaching Clinic

THE USE OF LABORATORY TESTS IN DIABETES

SIMPLE laboratory tests are essential for the diagnosis and for the management of diabetes mellitus. The use of such tests in the management of this disease will be examined first. In doing this I should like to take as a text this statement: "The novice lends too much credence to the test."

The laboratory tests which appear most relevant to the care of the diabetic patient are simple to perform. Enough is known of their physiologic meaning so that their practical application is relatively easy. This happy state of affairs still demands that the physician know something about diabetes and diabetics. The novice lacks this knowledge and consequently lends too much credence to the results of the tests.

Colwell's (1) monograph contains a table of the classification of diabetes into four main types or degrees, namely, improved; mild; severe; and, with acute complications. Having borrowed the dignity of Colwell's classification, it has been oversimplified in Table 1.

TABLE 1. TYPES OF DIABETIC PATIENTS

1. The thin, young patient with severe, labile diabetes.
2. The obese, elderly patient with mild, stable diabetes.
3. The diabetic with complications:
a. Infectious
b. Atherosclerotic
c. Miscellaneous.
4. The diabetic with impending acidosis.

Although there are exceptions to these categories, their simplicity may be of service to general physicians. If one begins by thinking in this way about diabetics, he will probably be able to recognize the exceptions, such as the occasional child with stable diabetes or the unusual elderly person with labile diabetes.

This abbreviated classification of diabetics will be used to illustrate the fact that different laboratory tests are needed in different circumstances.

The classification itself has been developed by the application of many tests to many patients. The young physician ought to repeat many of these experiences for himself. For this reason, the beginner may use it to accelerate the conclusions drawn from laboratory tests, since the table shows what these tests may reveal. The older physician, who has learned some such outline by his own experience, is more likely to dwell on the exceptional patients which he observes. The following discussion may help in acquiring, but will not substitute for, a knowledge of what each test means and a comprehension of different patients; these can only be learned by careful observation. We shall deal only with the first three categories—the most commonly observed types of diabetes.

I. *What laboratory tests are useful in young patients with severe diabetes?*

The glucose tolerance test? No. The diagnosis is usually obvious in these persons with rapidly developing, severe diabetes. Confirmation of hyperglycemia, to explain the glycosuria and to rule out other melliturias is ordinarily all that is needed.

Qualitative urinary sugar? This has some uses which are not always understood.

a. It reminds me to cite Joslin's valuable dictum that all glycosuria is diabetes until proven otherwise; but the proof of diabetes by the demonstration of hyperglycemia has previously been noted.

b. As a guide to the management of diabetes, the qualitative test has little use in this group of patients. Few children will remain free of glycosuria throughout the day unless they also suffer prohibitive insulin reactions. My definition of a labile diabetic is one who has a small margin of safety between freedom from glycosuria and an insulin reaction. In view of this small margin, the mere presence of glycosuria is of little help. Under some circumstances the test is useful. Thus, when a urine collection representing a long period (such as overnight) is free of sugar, the test has an approximate quantitative significance. If a patient is controlled to the extent of being free of glycosuria for a third, a half, or three quarters of the twenty-four hours, the care is good or excellent, and the time when insulin or diet should be further adjusted is indicated.

In connection with the qualitative test for glycosuria a word is in order about the effort to read the results of these tests on an arbitrary scale of 1+ to 4+ and give them quantitative meaning. Anyone who has compared qualitative and quantitative tests on the same sample of urine knows that this use of plus signs is an unreliable procedure. Numerous physicians have observed that the use of Benedict's qualitative solution as a quantitative method of measuring glycosuria by recording the results in plus signs is grossly inaccurate. The errors due to disregarding the wide

range of urine volume and the errors dependent on acidity or alkalinity of the urine are noted. I have observed these features of the test and agree with the warning. The same difficulties seem to apply to clinitest® and other modern reagents. Knowing these things, one can still make a proper use of these serial qualitative tests. They show 1) when qualitative control of glycosuria is being secured, and 2) the time of day when more insulin may be needed. They also may have a quantitative meaning which is overlooked in the strict criticism of the chemist. If the samples are taken from the total quantity of urine voided between specified hours, one learns for how many hours of the day the patient is sugar-free. If, for example, the tests show that for fourteen to eighteen hours of the day there is no glycosuria, control has progressed fairly well. When using these spot tests, it is to be remembered that the 24-hour insulin requirement and the consequent daily utilization of carbohydrate are the fundamental considerations, which are more important than the finding of a single four-hour or six-hour period of glycosuria.

Quantitative urinary sugar? The lability or metabolic irregularity noted in the young patient with severe diabetes makes the quantitative assay of urinary sugar valuable and, at times, a necessity. This determination reveals the true meaning of the qualitative measurements of a glycosuria which resists the usual efforts at control. In unstable diabetics and in diabetics whose intake of food and insulin is made irregular by surgery or complicating disease, it is often helpful to know the amount of carbohydrate utilized. If the difference between the intake and excretion of carbohydrate amounts to 100 grams or more daily, the patient should be safe, except in unusual circumstances. When I see a labile diabetic whose daily glucose excretion varies from 5 to 80 grams on a constant hospital regimen but in whom the quantitative tests show that, in spite of this glycosuria, enough glucose is utilized to assure health and growth, I cease to scold the patient for the qualitative glycosuria which is found on clinic visits. It is astonishing to see the number of physicians who are confused about this type of glycosuria. It can easily be interpreted when the measurements are made on a quantitative basis.

Acetone test? The use of modern home tests for urinary acetone, which are also applicable to serum or plasma, may be of service. In the case of the patient with severe labile diabetes, it is necessary to know the metabolic pattern. Some diabetic children have slight acetonuria once or twice a day; this represents slight difficulty in timing the action of insulin. If control for the whole day is good, such acetonuria may be disregarded and the patient ought not to be worried about it. On the other hand, it is desirable that every diabetic test the urine for acetone when intercurrent

illness develops. The presence of marked (4+) acetonuria will be a warning to call the doctor, take more insulin and otherwise act to forestall acidosis.

In the diabetic, as in the normal person, acetonuria without glycosuria can usually be disregarded. Without glycosuria, the physiologic ketonuria of such conditions as fasting fever, or hyperthyroidism is harmless.

Blood sugar level? In the patient with severe, labile diabetes it may be possible to work out the pattern of the blood sugar concentration during the day, but this is a long and tedious procedure (2a). In most instances, the blood sugar level will appear to be an erratic, widely fluctuating value which gives no information about what may happen at the same time on another day. In recent years I have paid little attention to the blood sugar level in this type of patient, and rarely perform this test in children. However, those who deal with intelligent and cooperative patients often use the concentration of blood sugar as the principal criterion of regulation. Thus, John (2b) writes: "For the evaluation of the patient's condition, we need three blood sugars a day, one before each meal, to guide us as to the needed changes in insulin therapy." He notes that if these three blood sugar values are normal there will be little or no glycosuria, but he also notes that in some patients glycosuria is still present, no matter what the routine, and any routine must then be a compromise. In his chapter on diabetes in children, he says almost nothing about the blood sugar level but emphasizes the value of quantitative determinations of urinary sugar. In any case, the labile diabetics are the ones who must be recognized as such in order that their laboratory tests may be adjusted to their special needs.

II. *What laboratory tests are useful in elderly patients with stable diabetes?*

These persons ordinarily have a wide margin of safety between freedom from glycosuria and insulin reactions. The doctor will, therefore, expect to keep such patients free of glycosuria—by diet if they are obese, and by diet plus 20–40 units of insulin if they are of proper weight and the diabetes more severe.

Qualitative urinary sugar? This is usually adequate to demonstrate the initial regulation, in stable diabetes. It measures the response to diet, and the adjustment of insulin to abolish glycosuria without insulin reactions. If these patients test their urine for sugar at home, the results will be a guide to management and a warning of acute complications. In both labile and stable diabetics, my usual rule is to request that they examine four samples of urine a day on one day a week. Diabetics must not be expected to spend their lives examining urine; only monomaniacs who

investigate diabetes do this. Diabetics must not be made "laboratory invalids." Each physician should individualize his advice on this point (3, 4).

Quantitative urinary sugar? This group of patients can usually be managed without quantitative measurement, although this type of assay may be used in anyone and it may be of value in occasional instances.

Blood sugar level? Determination of the level of the blood sugar is essential to the diagnosis of diabetes. Many kinds of screening tests may be used. The conventional glucose tolerance test is still the most exact. A single blood sample taken two hours after ingestion of 100 grams of glucose is recommended by Duncan (5). A blood sugar determination after any full meal is the most easily available of all rapid screening methods but its interpretation is less accurate. At a recent conference the outline in Table 2 was accepted by several authorities.

TABLE 2

Test	Highest normal blood sugar values		Rating, if above normal	Interpretation
	Time (hrs.)	(mg./100 ml.)		
Oral glucose, 100 Gm.	0	110	1	If any two of the three ratings are abnormal, the patient is considered diabetic (*see* text)
	1	170 or 120	1	
	2			
	3	110	1	

The blood sugar values are those obtained by the Nelson-Somogyi method. The use of one-hour and/or two-hour samples to give one rating indicates that these values are regarded as less important than the fasting and three-hour values. All consultants agreed that the return of the blood sugar to a normal level in three hours is the most important feature of the test.

In the control of diabetes, the blood sugar test should be used after the urine is sugar-free. This will provide good regulation of the patients with a high renal threshold for glucose. One may follow John's (2b) method of determining the blood sugar level three times a day before meals on the days when the patient is seen by the physician, or use a modification of this procedure. The point is that blood sugar determinations aid in the more accurate control of diabetes after the urine is largely sugar-free. A single blood sugar value may be regarded as a one-minute point on a 24-hour curve. As a rule, blood sugar determinations are made in order to

learn the highest or the lowest level reached during the day. The exact times of interest to the physician will depend upon the patient and the type of insulin which is being used.

Acetone test? If stable diabetics were to use the acetone test for all intercurrent illnesses, they would be more alert to the need for extra care at such times. They would gradually learn the difference between the overeating which causes glycosuria only, and the illnesses which cause glycosuria and perhaps acetonuria even if less food than usual be taken. Although endorsing the educational value of the test in these patients, my first recommendation would be that every diabetic should have a clinical thermometer.

III. *What laboratory tests are useful in the diabetic with acute complications?*

By acute complications is meant any infectious, traumatic, surgical or other injury of tissue sufficient to cause a systemic metabolic reaction. This metabolic reaction to injury is characterized by relative insulin resistance and by a wide margin between freedom from glycosuria and insulin reaction, even in previously labile diabetics. Therefore, in this category of diabetics with acute complications, a single common description will serve, if one remembers that with the subsidence of complications, different laboratory tests will again be needed.

Qualitative urinary sugar? This test is useful in the milder or more chronic complications, during which the diabetes can be controlled with additional insulin. However, in severe, acute disorders there is likely to be such disruption of the patient's regimen that glycosuria will only indicate trouble without revealing its degree. Intravenous glucose almost always causes glycosuria in normal as well as in diabetic patients, and this renders qualitative urinalysis useless.

Quantitative urinary sugar? Although the febrile diabetic has a wide margin of safety, the irregularity of emergency illness makes it desirable to know that enough glucose is being utilized to tide the patient over the crisis. The period of worst stress is usually of brief duration; the urine will not be sugar-free, either because of intravenous glucose, or because of lack of time to adjust the insulin dosage, or both. In these periods of pressure a record of the intake and excretion of glucose in grams per day is most useful. In general, 150 grams per day for a febrile adult should permit adequate nutrition and good healing and should prevent ketonuria.

Acetone test? This is valuable and should be performed daily or several times a day in patients with severe diabetes and complications. The prepared powders add to the ease with which the test can be made directly at the bedside. It should be carried out in conjunction with quantitative

urinary glucose measurements, in order to ascertain that the amount of glucose utilized and the insulin dose are regulated in such a way as to control ketogenesis. In severe fevers, some ketonuria may occur even in the absence of glycosuria. In such a situation the diabetic will fare no worse than the non-diabetic.

Blood sugar level? In the majority of severe complications the use of surgery or of antibiotics makes the period of metabolic disturbance brief—a matter of a few days. Attempting to achieve accurate regulation of the blood sugar level during such a short disturbance is hardly worth the effort. Between the effect of intravenous glucose and that of the rapidly changing condition, the blood sugar value is not likely to add much information. If there is prolonged fever or prolonged healing of some lesion without fever, the diabetes can be controlled by making the urine free of sugar and then using the time in the hospital to see that the blood sugar level is normal several times during the day. This may require more insulin than the patient will need after recovery.

TABLE 3. SUGGESTED VALUE OF SIMPLE TESTS IN DIABETES

Type of diabetic patient	Urinary Sugar		Urinary Acetone	Blood Sugar Level
	Qualitative	Quantitative		
1. Labile	Limited value (timing of samples)	Great value	Education. Warns of infection	Little use after diagnosis (exceptions)
2. Stable	No glycosuria ("margin of safety")	Rarely needed	Warns of infection	Required after control of glycosuria
3. Type 1 or 2 with fever, etc.	Diagnosis. Often regulates ("margin of safety")	Great value in irregular metabolism, i.v. glucose, etc.	*Essential*	Omitted in brief emergency. Required in chronic fever.

In Table 3 an attempt has been made to summarize the relation of four simple laboratory tests to the most commonly observed types of diabetic patients. It merely indicates some of the facts which have been discussed, but it may illustrate the only conclusion which I wish to make. This conclusion is that from among all the laboratory tests which may be used in diabetes, one ought to select and evaluate those which are of most use in a particular patient. The novice believes in the test instead of using wisdom in the selection of the test. Not all senior physicians would concur

with everything I have said, but I am sure they would agree that the laboratory test should be appropriate to the patient's condition.

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Letters to the Editor

THYROXINE-BINDING BY SERA OF PREGNANT WOMEN*

To the Editor:

Alterations in the metabolism of iodine during pregnancy have aroused the interest of many investigators. Enlargement of the thyroid gland, elevation of the basal metabolic rate, and increase in the concentration of the circulating thyroid hormone have been observed, in the absence of clinical evidence of hyperthyroidism (1). Augmentation of thyroidal accumulation of radioiodine during pregnancy has been noted (2), although few observations have been made, owing to general avoidance of administering radioactive isotopes to pregnant women. The mechanisms underlying these physiologic alterations are unknown.

In serum obtained from pregnant women at term, Longsworth, Curtis and Pembroke (3) observed a reduction in the concentration of albumin of about 1 gram per cent, a slight increase in the concentration of alpha-1 globulins, and a striking increase in the concentrations of alpha-2 and beta globulins, compared with the concentrations of these components in serum obtained from nonpregnant controls.

Since Gordon, Gross, O'Connor and Pitt-Rivers first described the migration of labelled thyroid hormone with an alpha globulin fraction of serum during paper electrophoresis (4), other investigators have further clarified relationships between thyroxine and the proteins of serum. It appears that thyroxine enters into a reversible binding equilibrium between the thyroxine-binding protein (TBP), albumin, and proteins of tissues in which the distribution of thyroxine is dependent upon the concentrations of the components in the system (5). Therefore, assessment of possible alterations in the binding of thyroxine by TBP in sera obtained from pregnant women seemed of particular interest.

Sera were collected from 9 nonpregant women in the child-bearing age (disregarding the phase of the menstrual cycle) and from 22 pregnant women; 6 in the first trimester, 8 in the second, and 8 in the third. The sera were frozen until used. Samples of serum were prepared with *l*-thyroxine-I^{131} and stable *l*-thyroxine in amounts sufficient to yield concentrations of

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iodine of 12.2, 46.5, 85.8, and 179.4 micrograms per 100 ml. The concentrations of thyroxine were verified by chemical analysis (SPI). Following electrophoresis of aliquots in filter paper, the distribution of radioactivity between TBP and albumin was determined. Preparative, electrophoretic, and analytic techniques have been described and evaluated in a previous communication (6).

At all concentrations of thyroxine, a marked difference in the distribution of radiothyroxine was noted between sera obtained from pregnant women and from nonpregnant controls (Table 1). In the pregnant group, the percentage of radioactivity associated with TBP was markedly increased, and the percentage associated with albumin correspondingly diminished ($P < .001$). Among samples of serum from pregnant women, statistically significant differences between trimesters could not be discerned ($P > .05$).

TABLE 1. DISTRIBUTION OF I^{131}-LABELLED AND STABLE THYROXINE ADDED TO THE SERUM OF PREGNANT AND NONPREGNANT WOMEN*

PBI added (μg./100 ml.)	Control		Pregnancy†		1st Trimester		2nd Trimester		3rd Trimester	
	% TPB	% Alb.	% TBP	% Alb.	% TBP	% Alb.	% TBP	% Alb.	% TBP	% Alb.
12.2	80.4±1.1	14.6±1.0	91.5±0.5	4.9±0.3	90.5±0.4	5.7±0.2	93.1±0.5	4.3±0.2	90.6±1.2	4.9±0.5
46.5	50.2±1.8	42.1±1.6	81.8±1.0	13.3±0.7	81.1±1.5	14.0±1.3	81.9±1.5	12.8±1.2	82.5±2.0	12.5±1.8
85.8	38.5±2.2	54.5±1.9	65.6±0.7	27.7±1.0	65.0±0.8	28.6±0.8	65.8±1.2	27.8±1.8	65.9±2.3	26.9±1.9
179.4	28.9±2.3	62.8±2.4	48.7±1.3	42.8±1.1	47.8±2.6	44.4±1.9	49.1±2.1	41.6±2.0	48.9±2.4	42.9±2.0

* Values represent mean±std. error for each group.
† Pooled values of all three trimesters.

The origin of this alteration in the transport of thyroxine remains obscure. Possibilities to be considered include a change in the concentration of thyroxine's principal carriers, TBP and albumin, or in their avidity for the hormone. Preliminary data indicate that when the concentration of albumin in the serum of pregnant women is restored to normal by *in vitro* supplementation, disproportionate binding of thyroxine to TBP persists.

Further studies of the causes and consequences of this alteration in the binding of thyroxine by sera of pregnant women are in progress.

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QUANTITATIVE URINARY 17-HYDROXYCORTICOID PATTERNS: EFFECT OF ACTH AND "OPERATIVE STRESS"*

TO THE EDITOR:

During preliminary studies of the relationship of cortisol metabolism to "surgical stress" it was observed that the stimulation of adrenal secretion by ACTH administration or by the more complex stress of general surgery was accompanied by changes in the quantitative excretion pattern of certain urinary steroid metabolites. We are presenting data which confirm the statement made by Cope and Hurlock (1) that after surgery or ACTH injection "a *tendency* can be observed for tetrahydro-compound F excretion to rise more than does tetrahydrocortisone." We wish to point out that this "tendency" is made evident by the rapid increase in the relative proportion of THF[1] to THE in the urine after such adrenal stimulation. The data presented here are representative of a larger number of similar observations. It is considered to be preliminary to a more detailed body of work to be reported at a later date.

Thirty-milliliter samples of urine were incubated with 30,000 Fishman units of beta-glucuronidase[2] at pH 5 and 47° C. overnight. The urine was then extracted three times with ⅔ volumes of freshly distilled chloroform,

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[1] Compounds referred to are the following: THF, tetrahydrocortisol (3α, 11β, 17α-21-tetrahydroxypregnan-20-one); THE, tetrahydrocortisone (3α,17α,21-trihydroxypregnan-11,20-dione); F, cortisol; E, cortisone.

[2] Supplied as "Ketodase," a soluble beef-liver preparation, Warner-Chilcott Laboratories, New York.

washed with ⅓ volume of 0.1 N NaOH, and ⅓ volume of water. The washings were back-extracted with equal volumes of chloroform. The total chloroform extract was dried over sodium sulfate and evaporated to dryness at 45° C. with the aid of a stream of air. Aliquots of the dried residues

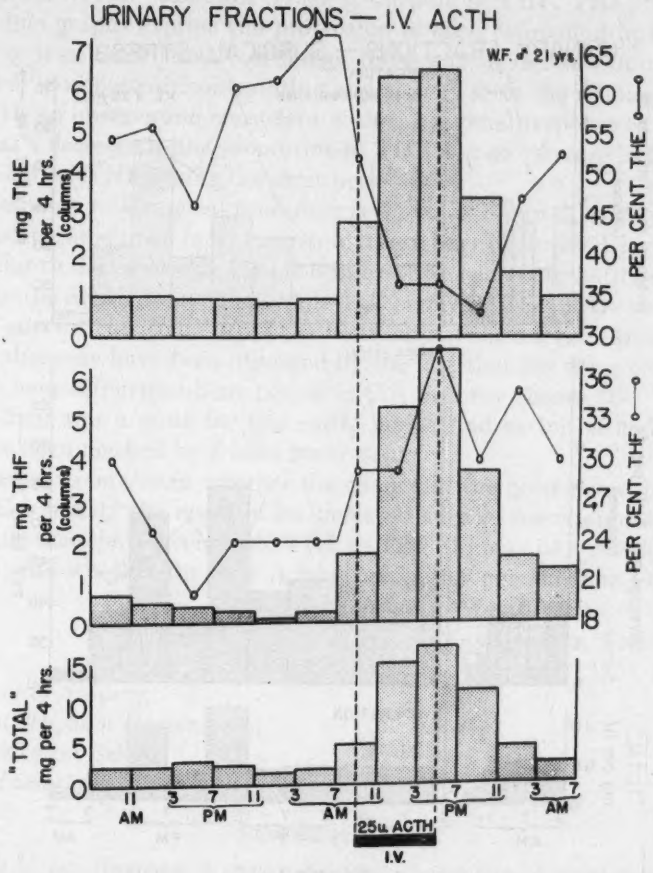
URINARY FRACTIONS — I.V. ACTH
W.F. ♂ 21 yrs.
mg THE per 4 hrs. (columns)
PER CENT THE
mg THF per 4 hrs. (columns)
PER CENT THF
"TOTAL" mg per 4 hrs.
11 AM
3 7 11 PM
3 7 11 AM
25u. ACTH I.V.
3 7 11 PM
3 7 AM

FIGURE 1

along with quantitative standards of THF, THE, F, and E were chromatographed in a toluene:methanol:water, 4:3:1, system at 35° C. for sixteen hours, according to the method of Bush (2). The chromatograms were then sprayed with alkaline Blue Tetrazolium[3] (BT), washed, and dried at room

[3] Obtained from Dajac Laboratories, Division of Monomer-Polymer, Leominster, Massachusetts.

temperature. The relatively insoluble blue formazan spots were excised and eluted with 1 ml. of 20 per cent ethyl acetate in pyridine.[4] The absorbance of the eluted formazan was read at 580 mμ against the respective standards, and quantitative values were thus obtained.

BT is reduced to a formazan by those steroids with C_{20}-ketone, C_{21}-

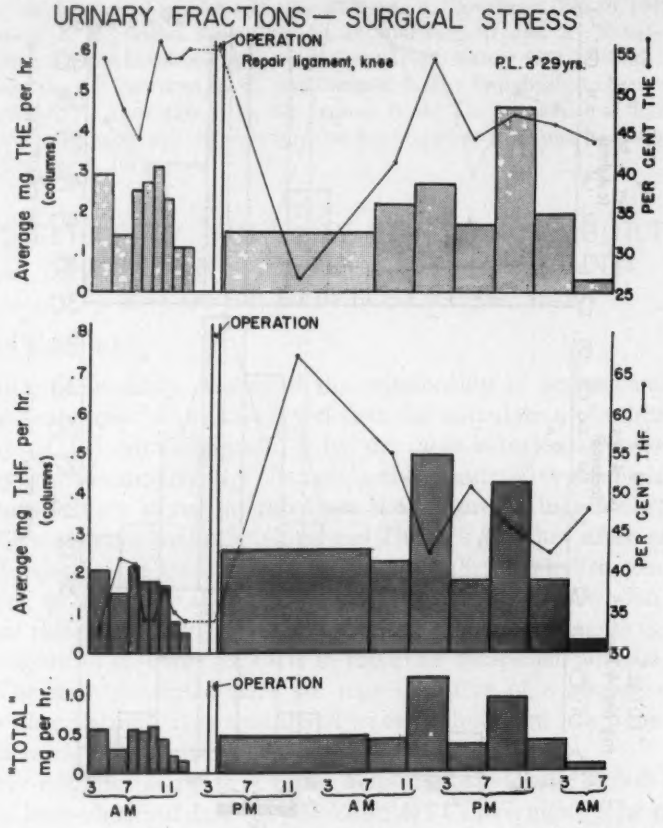
URINARY FRACTIONS — SURGICAL STRESS
OPERATION
Repair ligament, knee
P.L. ♂ 29 yrs.
Average mg THE per hr. (columns)
PER CENT THE
OPERATION
Average mg THF per hr. (columns)
PER CENT THF
OPERATION
"TOTAL" mg per hr.
3 7 11 3 7 11 3 7 11 3 7 11 3 7
AM PM AM PM AM

FIGURE 2

hydroxyl groups. The group of compounds evaluated were those with polarities greater than E and equal to or less than THF. They were identified mainly by position on the chromatogram and include the following compounds in order of decreasing polarity: THF, "X" (an unidentified compound), THE, and F. Amounts of E present were usually less than 5 per cent of the total of the foregoing compounds.

[4] Suggested by Dr. L. Engel, Harvard Medical School.

Figure 1 illustrates the changes in the relative proportions of THE and THF excreted in the urine of a 21-year-old normal male volunteer, before, during and after intravenous administration of 25 units of ACTH. The bargraphs indicate the urinary excretion per unit time of either THF, THE or the "Total" excretion which is the sum of THF, THE, "X" and F. The line graphs express the proportion of each compound in terms of the per cent of the "Total." Although there was an increase in excretion of "Total" adrenal corticoids during and shortly after the administration of ACTH, an observation consistent with the adrenal response to ACTH, there was a decrease in the proportion of THE and an increase in the relative amount of THF during the same period.

The response to a surgical procedure is shown in Figure 2. A 29-year-old male was operated upon for the repair of a torn knee ligament. His response was similar to that shown in Figure 1, in that the relative quantity of THE fell after the stress or trauma while the percentage of THF rose. The "Total" adrenal corticoid output per hour did not show a rise. An increase in this value may have been obscured by the fact that the urine collection occurred over a fourteen-hour period in this instance; hence the "Total" mg. per hour was a *mean* for this entire period and an initial high value may have been masked by a later lower value.

It remains to be shown whether the change in the proportions of THF and THE is merely the result of an increased adrenal secretion of cortisol (suggesting that the conversion of THF to THE (3) may be a rate-limiting reaction), and whether the liver is involved in this phenomenon (4, 5, 6).

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FLUCTUATION IN THE EXCRETION OF ADRENOCORTICAL STEROIDS IN A CASE OF CUSHING'S SYNDROME

To the Editor:

We have found enormous variations in the excretion of 17-ketosteroids and of 17-ketogenic steroids in an unmarried woman, aged 40, exhibiting all the classic symptoms of Cushing's syndrome. The diagnosis, adrenocortical hyperplasia, was verified on a subsequent pathohistologic examination of the adrenals removed at operation. Previously, in 1952, she had undergone bilateral oophorectomy on the grounds of polycystic ovaries. It can therefore be assumed that all the urinary steroids excreted by the patient were of adrenocortical origin.

17-Ketosteroids were estimated according to the micromethod described by Vestergaard (1) and 17-ketogenic steroids according to a slightly modified Norymberski technique, as reported previously (2). The daily excre-

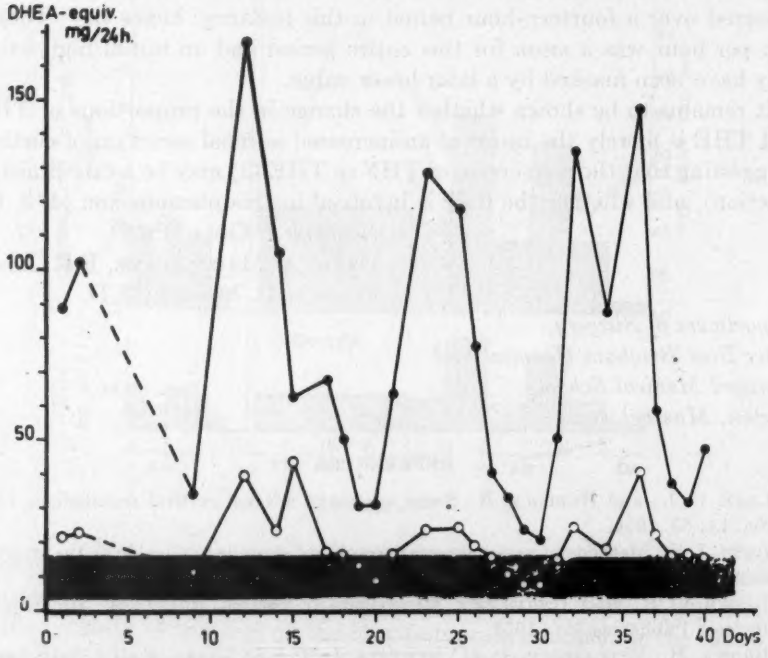

Fig. 1. Day-to-day variation in the excretion of 17-ketosteroids (open circles) and 17-ketogenic steroids (filled circles) in a 40-year-old woman with Cushing's syndrome. Shaded area indicates normal range for 17-ketosteroid excretion of apparently healthy women in the same age-group.

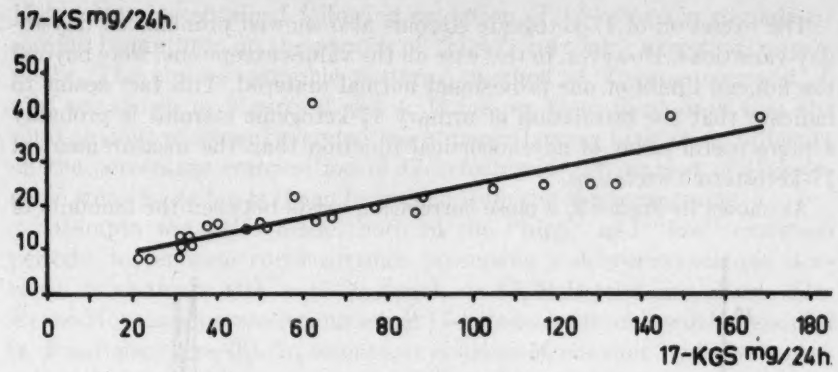

FIG. 2. Correlation between the daily excretion of 17-ketosteroids and 17-ketogenic steroids. The equation of the line is: $Y = 5.7 + 3.36x$. From the data, a coefficient of correlation ($r = 0.76$) was calculated.

tion of these steroids was followed for approximately one month, as shown in Figure 1. It appears from these data that the excretion of 17-ketosteroids showed pathologically high peak-values followed by apparently normal values. The excretion values would seem to indicate rather rhythmic variations. Similar fluctuations in the excretion of 17-ketosteroids have been reported by Zimmermann (3) and by Wilkins (4).

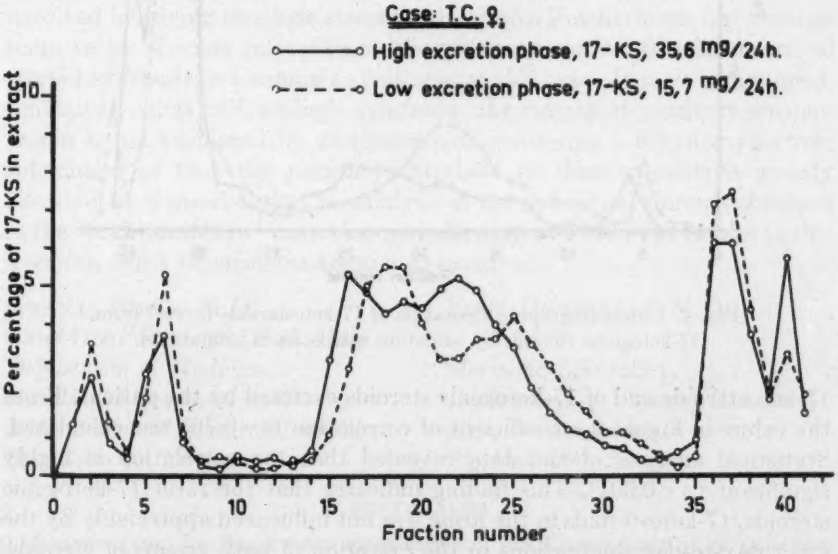

FIG. 3. Chromatographic pattern of 17-ketosteroids excreted during different phases.

The excretion of 17-ketogenic steroids also showed pronounced day-to-day variations. However, in this case all the values except one, were beyond the fiducial limits of our provisional normal material. This fact seems to indicate that the estimation of urinary 17-ketogenic steroids is probably a more useful index of adrenocortical function than the measurement of 17-ketosteroid excretion.

As shown in Figure 2, a close correlation exists between the amounts of

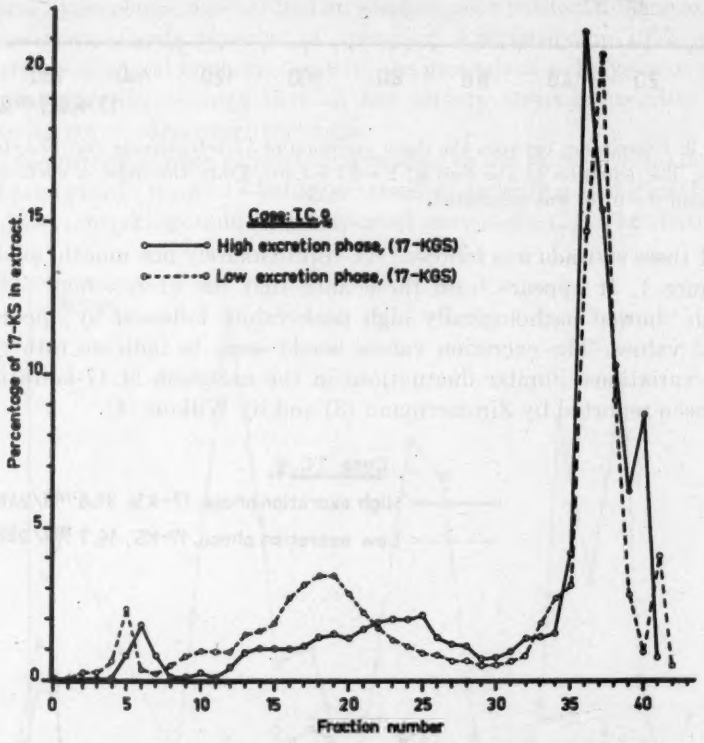

FIG. 4. Chromatographic separation of 17-ketosteroids formed from 17-ketogenic steroids by oxidation with sodium bismuthate.

17-ketosteroids and of 17-ketogenic steroids excreted by the patient. From the values in Figure 2 a coefficient of correlation ($r = 0.76$) was calculated. Statistical analysis of the data revealed that the correlation is highly significant ($p < 0.001$). This finding indicates that the ratio: 17-ketogenic steroids/17-ketosteroids in the urine was not influenced appreciably by the great day-to-day fluctuations in the excretion of both groups of steroids.

Efforts were made to study the pattern of 17-ketosteroids, and also of

17-ketosteroids obtained following oxidation of 17-ketogenic steroids by sodium bismuthate, in the periods of "high" and "low" excretion, respectively. The chromatographic patterns (method of Zygmuntowicz *et al.* (5)) are shown in Figures 3 and 4. It follows from these data that the total amount of steroid excreted has apparently very little or no influence on the percentage composition of 17-ketosteroids, or on that of 17-ketogenic steroids, as far as it can be judged from the chromatograms.

Attempts were also made, both in the "high" and "low" excretion periods, to separate corticosteroids possessing a dihydroxyacetone side-chain from those with a 17-20-glycol, or 17,20,21-triol side-chain. This method for the separate estimation of 17-ketogenic steroids will be reported in detail elsewhere (6). In essence, it consists of enzymic hydrolysis by a spleen β-glucuronidase preparation followed by repeated treatment with Girard's reagent T. The ketonic as well as the non-ketonic fractions respectively are oxidized by sodium bismuthate and the individual steroids are separated by chromatography on alumina. Under our experimental conditions only very small amounts, if any, of 11-ketosteroids will form complexes with the Girard-reagent (7).

Using this method of separate analysis of various 17-ketogenic steroids, all our efforts to demonstrate any major difference in the composition of 17-ketogenic steroids found in the "high" and "low" excretion periods, met with a complete failure.

We believe that the present data point to the great diagnostic hazards involved in relying on single steroid estimations. Furthermore, our findings seem to be of some relevance to the pituitary control of adrenocortical steroid synthesis in Cushing's syndrome of this type. It may be assumed, tentatively, that in Cushing's syndrome the release of pituitary corticotropin is not regulated by corticosteroids possessing a dihydroxyacetone side-chain, or that the pituitary threshold to these steroids is greatly elevated. It is possible that an analysis of the individual steroids obtained in the "high" and "low" excretion periods, may provide some answer to this question. Such experiments are now in progress.

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COMMENTS ON RAT HYPEREMIA PREGNANCY TEST

To the Editor:

In 1940, a series of experiments established the use of the female rat as a completely reliable test animal for the diagnosis of pregnancy. This procedure was described by Frank and Berman in 1941 (1) and was the first successful pregnancy test utilizing rats which provided results within eight to twenty-four hours. The test involves two subcutaneous injections of 5 ml. of untreated, centrifuged urine into 2 immature 50-Gm. female rats (weight range, 45–60 Gm.) and sacrifice of the animals with illuminating gas the following morning, within sixteen to twenty-four hours after the first injection. Examination of their ovaries is made by gross inspection with the naked eye. A characteristic hyperemia of the ovaries is induced by pregnancy urines. A full discussion and details of the test, as well as an outline of the indispensable precautions have been published recently (2). In the hands of the author, over 9,000 tests have yielded results which are 100 per cent accurate in the 16–24 hour period.

Our report stated that results obtained in eight hours are reliable only if positive. We studied all dose ranges of urine, tried both untreated and extracted urines, tried various sized animals, used all injection routes (intravenous, intraperitoneal and subcutaneous) and sacrificed animals at varying time intervals. We were able to obtain results in as short a period as one-half hour after injection. In answer to a query from Zondek, we reported in 1942 (3): "Even in four hours, positive reactions have been obtained but further study will be necessary before the reliability of these shorter observations will be considered trustworthy." Since we obtained so

many false negatives with known pregnancy urines, we did not proceed further with this work and did not deem our results worthy of publication.

There have been many modifications offered by other investigators since the Frank-Berman rat hyperemia pregnancy test was reported. These modifications involve changes in dosage of urine, number of rats, weight of rats, injection route and time of sacrificing animals. No modification has improved on the accuracy of the Frank-Berman test; rather, the modifications have introduced a high percentage of inaccurate results.

There have been several misconceptions about the Frank-Berman pregnancy test. Amoral (4) states that we use 3 rats weighing 40 grams each and that we filter the urine before use. We stated that we use 2 rats weighing 50 grams each and that we centrifuge the urine. Fried (5) states that we increase the amount of urine injected into rats when we use daytime specimens. We never vary the dose, always injecting a total of 10.0 ml. of urine. Merchante (6) states that we inject 1 rat intra-abdominally with 5.0 ml of urine, sacrificing it in eight hours; and that we inject another rat with 10.0 ml. of urine, sacrificing it in eighteen hours. We inject 2 rats with 10.0 ml. urine each, subcutaneously, and sacrifice the animals in sixteen to twenty-four hours. We noted that results were obtainable in eight hours in rats injected subcutaneously with 10.0 ml. of urine, but that the value of the eight-hour test was limited because negative results at eight hours could not be relied upon as truly negative. We do not use the eight-hour test as a routine procedure. Hoffman *et al.* (7) report that 3.8 per cent of rats gave us false negative results when we said that "*it is imperative to use 2 rats for every test*, as at least 3.8 per cent have proved refractory" (1). Salmon *et al.* (8) and Zondek *et al.* (9) failed to mention that we had previously reported results obtainable within eight and four hours.

Zondek *et al.* (9) in 1945 published an article on the 24-hour rat hyperemia pregnancy test using a dosage of 4.0 ml. of urine. They stated: "When, for example, we injected 10 cc. of pregnancy urine, as proposed by Frank and Berman, we obtained false positives, from which we concluded that the dose was too high." This statement is not consistent with the facts, since pregnancy urines elicit a positive reaction in the Frank-Berman test, but these are not false positive responses. After our 1941 paper appeared, Zondek wrote to us (December 31, 1941) to advise that he previously had attempted to shorten the pregnancy test by methods similar to the Frank-Berman technique but that his efforts were nullified by the finding that in uterine fibroids, amenorrhea, menopausal castrates and carcinoma, the increased follicle-stimulating factor caused false positive reactions. Our published reply (3) advised that we had no such experience and found our results completely reliable. Since the hyperemia reaction is elicited only with chorionic gonadotropin, it is difficult to under-

stand how the urines in the cases mentioned by Zondek, which do not have chorionic gonadotropin, could yield positive pregnancy test results. No investigator to date has even reported finding the follicle-stimulating factor (anterior pituitary gonadotropin) in any type of case in a bio-assay on the rat using urine in the amount of 10.0 ml. Chorionic gonadotropin is found *only* in pregnancy, hydatidiform mole and chorionepithelioma (10).

It is hoped that this letter may correct some published misconceptions regarding the Frank-Berman pregnancy test.

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EFFECTS OF CHLORPROMAZINE ON PITUITARY FUNCTION

TO THE EDITOR:

In the course of treatment of mentally ill patients with chlorpromazine, it was observed that amenorrhea is frequently induced. The nature of this amenorrhea has been investigated.

Group I. Eleven mentally ill females were treated with chlorpromazine parentally in amounts ranging from 150 to 200 mg. per day for periods of three to fifteen weeks. Amenorrhea developed in 6 of these patients, in 3

of whom spontaneous galactorrhea also occurred. Vaginal smears indicated estrogen deficiency of various degrees in all 6 cases of amenorrhea; urinary FSH titration showed normal values of 15 to 30 m.u. per liter, and 17-ketosteroid excretion was within normal limits.

Group II. Nine patients, 5 males and 4 females, received chlorpromazine in daily doses of 350 to 400 mg. parenterally for ten days. At the end of this period, the 4 females showed estrogen deficiency in the vaginal smear and, subsequently, amenorrhea. In 3 of them a copious galactorrhea also developed. Urinary FSH on the tenth day of treatment in both males and females had increased from normal initial values of 15–30 m.u. per liter, to 66 m.u. per liter (1 case) and over 111 m.u. liter (7 cases). Three weeks after termination of treatment, the urinary FSH values had dropped to 10 m.u. per liter in the 3 cases studied. The galactorrhea had diminished considerably. The urinary 17-ketosteroid excretion in 4 of the male patients on the tenth day of treatment was found to be fairly low (5.9 to 9.6 mg. per twenty-four hours).

In this second group of cases a state of drowsiness, recollection of lost experiences and vivid dreams were observed during the short course of treatment. In several instances, when the dosage was 800 mg. daily, there was sleeplessness and psychologic agitation. In male patients there was decreased libido during prolonged treatment. The psychic changes and effects on behavior and sleep point to a hypothalamic effect of chlorpromazine. The degree of pituitary derangement seemed to bear some relationship to the dosage and duration of chlorpromazine administration. The nonpuerperal galactorrhea associated with high FSH excretion serves as proof of the independence of LTH and FSH factors.

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Acknowledgment

We wish to thank Dr. F. Sulman for several FSH titrations, as well as Dr. Herz, Miss V. Krauss, Messrs. N. Hazan and H. Gross for their assistance in the laboratory studies.

CONCERNING THE TERM "PSEUDO-PSEUDO-HYPOPARATHYROIDISM"

To the Editor:

One of the factors which hinder the better understanding of diseases is confusing nomenclature, and the term "pseudo-pseudohypoparathyroid-

ism" employed by Albright *et al.* in 1952 (1) is undoubtedly a case in point. A short reference may clarify this. In 1942 Albright *et al.* (2) described "pseudohypoparathyroidism," characterized by clinical and humoral manifestations of hypoparathyroidism caused by disturbances of the parathyroid end-organ, particularly the renal tubules. Along with these symptoms are others which are not constant, and are apparently independent of the parathyroid function (3)—abnormal formation of the face and skeletal segments (chondrodystrophia or dyschondroplasia) and foci of ectopic calcification.

So-called "pseudo-pseudohypoparathyroidism" (1) is characterized by the foregoing abnormalities of body-build, but with no clinical nor humoral manifestations of hypoparathyroidism, Similar cases have been recently reported by Miles and Elrick (4) and Roche (5). The patient studied by Roche presented only short stature, round face and shortening of the second, third and fourth metacarpals; the roentgenograms revealed exostoses with secondary metaphyseal dysplasia in both femoral necks, lower femoral metaphyses, upper tibial metaphyses, radii and ulnae. Response to the Ellsworth-Howard test was said to be slight in the case of Albright *et al.* (1), normal in the case of Miles and Elrick (4) and sluggish in the case of Roche (5), although it should be pointed out that the meaning of this test in normal persons has been seriously challenged by Milne (6), Dent (6) and Macgregor and Whitehead (8).

Hence we consider that the term "pseudo-pseudohypoparathyroidism" should be discarded, as it is complicated and arbitrary. It should be replaced in each case by the respective descriptive denomination: dyschondroplasia or exostoses with metaphyseal dysplasia or congenital skeletal malformations.

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The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7-8-9, 1956.*

The Committee on Local Arrangements is Dr. Norris J. Heckel as Chairman with Drs. M. Edward Davis, Sam Taylor, J. P. Greenhill, and Harley E. Cluxton as members of the Committee.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Sey-

mour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1956 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, May 3, 4 and 5, 1956.

THE AMERICAN GERIATRICS SOCIETY

The next Annual Meeting of the American Geriatrics Society will be held in the Palmer House, Chicago, Illinois, May 3 and 4, 1956.

MALFORD W. THEWLIS, *Secretary*,
25 Mechanic Street,
Wakefield, R.I.

FOURTH INTERNATIONAL CONGRESS OF INTERNAL MEDICINE

The Fourth International Congress of Internal Medicine will be held in Madrid, Spain, *September 19-23, 1956*, under the presidency of Prof. C. Jiménez Díaz.

There will be two main subjects, namely, "The Role of the Adrenals in the Pathogenesis and Evolution of Internal Diseases" and "Bronchial Asthma and Emphysema." Communications of these themes or on any others of free choice will also be admitted.

All inquiries should be addressed to the Secretariat: Drs. J. C. de Oya and J. Gimena, Hortaleza 90, Madrid, Spain.

REGIONAL MEETING—INTERNATIONAL FERTILITY ASSOCIATION

A regional meeting of the United States division of the International Fertility Association will be held at the Schenley Park Hotel, Pittsburgh, Pa., on *March 14, 1956*. Interesting scientific papers on infertility will be presented, and there will also be a panel discussion. All physicians are invited. For further information write to Maxwell Roland, M.D., Chairman, Liaison Committee, 1141 Eastern Parkway, Brooklyn 13, N. Y.

BOOK REGISTER

Die Überfunktion der Nebennieren. By RUDOLF CHWALLA. Hyperadrenalism (cortical and medullary) is defined and chapters are devoted to pathology, physiology, diseases, and interglandular relationships; 350 pages; 12 tables; 1955. Wilhelm Maudrich, Vienna. Sole representative in U.S.A. and Canada, Intercontinental Medical Book Corp., New York 16, N. Y. Price $11.52.

Growth at Adolescence. By J. M. TANNER, M.D. (Penna.), D.P.M. (London), Senior Lecturer in Physiology, St. Thomas's Hospital Medical School, University of London. A book addressed to pre-clinical and pediatric teachers, to biologists, to anthropologists, and above all to physicians concerned with children and adolescents; 224 pages; 11 plates; 57 figures; 12 tables; extensive bibliography; 1955. Charles C Thomas, Publisher, Springfield, Illinois. Price $6.50.

Psychiatric Research Reports. 1. A report of the American Psychiatric Association, edited by Members of the Committee on Research, 1954–55, concerning products recently introduced in the treatment of psychiatric disorders. The papers were presented at a Regional Research Conference held under the joint auspices of the American Psychiatric Association and the University of Texas, Medical Branch, Galveston, Texas, February 18–19, 1955; 158 pages; tables and figures; 1955. American Psychiatric Association, Washington 6, D. C. Price $2.00.

Scintography, 1955–1956. A collection of scintograms illustrating the modern medical technique of *in vivo* visualization of radioisotope distribution; 96 pages; 88 case histories with scintograms; 1955. R-C Scientific Instrument Co., Inc., Playa del Rey, California. Price $2.00.

The Clinical Care of the Diabetic. By JAMES J. SHORT, M.D., Associate Professor of Medicine, College of Medical Evangelists; Senior Attending Physician, Los Angeles County General Hospital, Los Angeles, California; 94 pages; various tables; 1955. San Lucas Press, Los Angeles, California. Price $3.95.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th St., New York 36, N. Y.

Administrative Medicine. Third Conference, October 6–8, 1954, Princeton, N. J. Edited by GEORGE S. STEVENSON, M.D., National and International Consultant, National Association for Mental Health, New York, N. Y. Deals with special characteristics of medical administration problems, including hospital and community relationships; 172 pages; 1955. Price $3.00.

Cybernetics. (Circular Causal and Feedback Mechanisms in Biological and Social Systems.) Tenth Conference, April 22–24, 1953, Princeton, N. J. Edited by HEINZ VON FOERSTER, Department of Electrical Engineering, University of Illinois, Champaign, Illinois, with the assistance of MARGARET MEAD, American Museum of Natural History, New York and HANS LUKAS TEUBER, Department of Psychiatry and Neurology, New York University College of Medicine, New York. Studies on activity of the brain, semantic information and its measures, meaning in language and how it is acquired, and a summary of points of agreement reached in previous nine conferences; 100 pages; 1955. Price $2.75.

Group Processes. First Conference, September 26–30, 1954, Ithaca, N. Y. Edited by BERTRAM SCHAFFNER, M.D., University Seminar on Communications, Columbia University, New York, N. Y. Deals with ontogeny and living systems, and studies of animal behavior; 334 pages; 40 figures; 1955. Price $5.50.